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## ODONTOGENIC TUMORS

### A CLASSIFICATION BASED ON OBSERVATIONS OF THE EPITHELIAL, MESENCHYMAL, AND MIXED VARIETIES \*

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#### INTRODUCTION

Odontogenic tumors, derived from dental or potential dental tissue, are common and have been discussed extensively in the literature. They have been classified as ameloblastomas, dentinomas, cementomas, and odontomas, each having been regarded as a distinct entity. There have been a few cases described in which one type arose in conjunction with another, but no report has suggested the influence of one tissue upon another in the pathogenesis of these tumors. The purpose of this report is three-fold: (1) to formulate a classification of odontogenic tumors in light of the large amount of material studied; (2) to describe their structure, especially that of the mixed group; and (3) to illustrate the inductive effects of one tissue on another in the production of odontogenic mixed tumors.

The material used in this study comprises 64 cases of odontogenic tumor selected from the files of the Registry of Dental and Oral Pathology and from the collection of one of us (K.H.T.). Twenty-six are chiefly epithelial tumors (Table I); 14, mesenchymal tumors (Table II), and 24, odontogenic mixed tumors (Table III).

#### EMBRYOLOGY AND HISTOLOGY OF THE TOOTH GERM

Since this study is concerned with tumors derived from dental tissues, a brief review of the normal embryology of the tooth seems essential. The precursor of the tooth bud appears as a thickening of the oral epithelium during the sixth week of embryonic life. This thickening is the primordium of the ectodermal portion of the tooth known as the dental lamina. The tooth bud, in the form of an invagination of the thickening, begins to take shape and the surrounding connective

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tissue differentiates into the dental papilla. Text-Figure 1 is a drawing of a tooth germ during the sixth month. The dental lamina is recognized at DL, while the permanent tooth bud is seen at A. At this stage the cells which form the tissues of the tooth have differentiated into the outer epithelial layer, the stellate reticulum, the stratum intermedium, the ameloblastic layer, the odontoblastic layer, and the dental papilla. Between the ameloblastic and odontoblastic layers are enamel and dentin.



Text-Fig. 1. A diagrammatic drawing of a tooth germ in the sixth month of embryonic life. Enamel and dentin have been laid down. DL, dental lamina; A, anlage of permanent tooth; OE, outer enamel epithelium; SR, stellate reticulum; SI, stratum intermedium; AM, ameloblastic layer; E, enamel; D, dentin; OL, odontoblastic layer; P, dental pulp.

#### CLASSIFICATION

We are in accord with the classification of odontogenic tumors based on structure; however, certain terms are used in this paper with modifications in meaning. "Odontogenic fibroma" is used to indicate the

soft mesenchymal tumor, while "dentinoma" is reserved for the pure mesenchymal tumor composed of islands of dentin in a connective tissue stroma. "Odontoma" is regarded as a mixed tumor made up of epithelial and mesenchymal elements and is spoken of as "odontogenic mixed tumor." Three types are recognized.

The following classification is the one adopted by us:

- I. Epithelial tumors
  1. Adamantoblastoma
  2. Enameloma
- II. Mesenchymal tumors
  1. Odontogenic fibroma
  2. Dentinoma
  3. Cementoma
- III. Odontogenic mixed tumors (odontomas)
  1. Soft odontoma—epithelium and mesoderm
  2. Soft and calcified odontoma—adamantoblastoma arising in conjunction with a forming or completely formed odontoma; all sorts of histologic variations due to the inductive effects of one tissue on another
  3. Completely formed odontoma with enamel, dentin, pulp, cementum, periodontal membrane
    - a. Compound (many small teeth)
    - b. Complex (irregular tooth structure)

Tumors may arise from either odontogenic epithelium or mesenchyma and may be either soft or calcified. The soft epithelial tumor is commonly called adamantoblastoma; the calcified, enameloma. The mesenchymal odontogenic tumor arises as a fibroma: in the calcified stage, if dentin is produced it is called dentinoma; if cementum, cementoma. Tumors may also originate from both the odontogenic epithelium and mesenchyma. These are known as odontomas or odontogenic mixed tumors and may belong to any one of three groups: (1) soft odontomas, (2) soft and calcified odontomas, and (3) completely calcified odontomas.

#### *Epithelial Tumors*

*Adamantoblastoma.* The adamantoblastoma is an epithelial odontogenic tumor in which the cells may differentiate to a variety of forms depending to some extent on the development of the epithelium at the time when tumor formation begins. Solid and cystic types are distinguished, both being of a slowly growing, benign nature, to judge from clinical observation. The frequency of recurrence after operation is due rather to the difficulty of removing the entire lesion by conservative operation than to any malignancy of the tumor; however, several cases recorded<sup>1, 2</sup> indicate that it can be malignant.

Microscopically, the tumor may appear solid, cystic, or a combination of both. If solid, it is composed of cords or strands of epithelium growing in a connective tissue stroma, or occasionally almost without

*Reprint*  
TABLE I  
*Adamantoblastoma*

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
1	69715	M	W	54	Noticed nodule 6 years previously, operated 5 years previously, swelling	Mandible	Cystic destruction from symphysis to angle of mandible
2	51505	M	W	24	Pain	Mandible	Dentigerous cyst involving 3rd molar and extending into ramus of mandible
3	75597	M	W	52	Oral-antral sinus, 25 years	Maxillary sinus	Opacity of sinus
4	72782	F	W	27	Swelling	Mandible	Cyst
5	75464	M	W	42	Large movable mass in tuberosity	Maxilla	
6	85184	M	W	36	Removal of dentigerous cyst 4 years previously	Mandible	Cyst in 3rd molar region, no tooth present
7	87202	M	C	21		Mandible	Cyst in 3rd molar region
8	86153	M	W	26	Removal of dentigerous cyst 4 years previously	Maxilla	Cystic lesion from central and incisor region to molar region
9	95817	F	C	37	24 operations performed during a period of 15 years (metastasized to lungs)	Mandible	Cystic lesion involving the body and ramus of mandible
10	108545	M	W	19	During a scuffle received a blow—pain and swelling	Mandible	Dentigerous cyst involving crown of 3rd molar, dentigerous cyst on opposite side
11	117002	M	W	21	Tenderness and swelling at angle of mandible of 48 hours' duration	Mandible	Dentigerous cyst—six other dentigerous cysts present in mandible and maxilla
12	117378	M	W	25	First noticed swelling 6 years ago—area incised and drained	Mandible	Multilocular lesion—no teeth present
13	120765	M	W	25	Swelling of 6 months' duration—no pain	Mandible	Multilocular lesion involving entire body of mandible and extending to inferior border
14	111372	F	W	2½		Mandible	Monocystic lesion
15	101010	M	W	36	Tumor first recognized 7 years previously	Mandible	Cyst in body of mandible
16	103102	M	W	25	Slight swelling, with mild dull pain, 2 years' duration	Mandible	Cyst in region of 2nd premolar and 1st molar

TABLE I (Continued)

Case No.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
17	109169	M	W	17	Previous operation, 3 teeth removed	Mandible	Multilocular cyst beneath 3rd molar
18	103313	M	W	23	Operation 5 years previously	Mandible	Multilocular cyst from canine region extending posteriorly into ramus—no teeth present
19	100372	M	W	31	2nd molar removed 4 years previously—roughening of gum 1 1/2 years previously	Mandible	Dentigerous cyst
20	94818	F	W	21	Swelling for 4 years, two operations	Mandible	Multilocular cyst
21	86845	M	W	28	Swelling	Mandible	Follicular cyst in 2nd and 3rd molar region
22	78638	M	C	25	Drainage of purulent exudate from area	Mandible	Dentigerous cyst involving crown of 3rd molar
23	66686	M	W			Mandible	
24	65091	F	W	60	3rd molar extracted, resulting in fistula into antrum	Maxilla	Radiolucent area in 3rd molar region
25	118679	M	W	32	Painless swelling, 10 years' duration	Mandible	Dentigerous cyst involving crown of canine with radiopaque masses in cystic portion
<i>Enameloma</i>							
26	H.M.G.	M	W	65	No symptoms	Mandible	Found on examination of autopsy specimen

\* Army Institute of Pathology.

stroma. The epithelial cords, which may form a network or take on a papillary structure, may resemble the anlage given off from the dental lamina and have a similar tendency to form small buds comparable with the earliest stage of the enamel organ. In the central portion of the cord or lobule the epithelium appears stellate, like the stellate reticulum of the enamel organ; in the periphery the cells are cuboidal to cylindrical.

The cystic type is characterized by lobules or strands of adamantine epithelium with a peripheral layer of cylindrical cells which, in structure, approach ameloblasts. These cells lie on a basement membrane. The central portion consists of stellate to squamous epithelium undergoing cystic degeneration. Pressure of cystic fluid causes these spaces

to enlarge; they often fuse and communicate with one another. Kronfeld<sup>8</sup> believed this kind of adamantoblastoma to be the result of progressive cyst formation in the solid type. He stated that the primary factor in the formation of cystic adamantoblastoma was degeneration of the stellate reticulum of the tumor, a concept supported by Robinson<sup>4</sup> and by data from this study.

The calcifying adamantoblastoma is rare. Kronfeld<sup>8</sup> stated that calcification was sometimes found, but never true enamel; and many other investigators have substantiated the fact that no enamel was produced in adamantoblastomas. In every case in our series the enamel-forming function was lacking, although the ameloblastic stage of development had been reached by groups of cells. Polarization of the ameloblasts was not encountered in adamantoblastomas but was seen in odontogenic mixed tumors.

The adenoid adamantoblastoma is the least common and is composed of small epithelial cells arranged in acini which usually contain a mucoid substance. The oral epithelium has potentialities of forming glandular as well as dental structures and, since the components of the enamel organ are derived from the epithelium of the oral cavity, cells may be present which have the ability to differentiate into adenomatous structures. Tissue of this kind composed one of the adamantoblastomas studied and was present in three of the odontogenic mixed tumors. The acinar arrangement was characteristic and the mucoid material filling the acini seemed to attract calcium salts. The globular pattern of the calcification suggested calcospherites.

The *enameloma* is a small tumor, sometimes situated between the roots of two teeth, but more frequently attached at the bifurcation of the roots of molars and premolars and at the cervical margin of single-rooted teeth. Sometimes spoken of as enamel drops or enamel pearls, enamelomas should be differentiated from small supernumerary teeth containing dentin and pulp. Microscopically, the enamel which appears as a space in the decalcified section is covered by a layer of atrophied epithelial cells, occasionally by a layer of cementum.

#### *Mesenchymal Odontogenic Tumors*

Odontogenic fibroma arises from the mesenchymal portion of the tooth germ, that is, from the embryonic tissue of the dental papilla or dental follicle, and, later, from the periodontal membrane. It may, therefore, occur in the coronal or apical regions of the tooth; if in the latter, it may be attached to the tooth and is often mistaken for a granuloma or an odontogenic cyst. One of us (K.H.T.)<sup>5</sup> has reported a case in which roentgenograms showed the tumor as a cystic

TABLE II  
*Odontogenic Fibroma*

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
27	M J	F	W	17	Toothache—swelling of gingiva	Mandible	Erupting 3rd molar
28	O P					Mandible	Large cystic area in periapical region of an impacted 3rd molar

*Cementoma*

29	80340	F	W	32	Tooth extracted and cementoma removed—recurred 5 years later	Mandible	Large cystic area in region of 2nd premolar, radiopaque mass in center
30	83048	M	W	32	Pain, lower molar	Mandible	Large radiopaque mass overlying root of 1st molar
31	95725	F	W		Routine roentgenologic examination	Mandible	Radiopaque mass lying between the roots of the 1st molar, surrounded by a translucent area
32	108526	M	W	43	Routine roentgenologic examination	Mandible	Radiopaque mass in the periapical region of the central incisor
33	121058	F	W		Routine roentgenologic examination	Mandible	Radiopaque mass rimmed by a translucent area in edentulous area, approximately region of 1st molar
34	121256	M	W	38	Routine roentgenologic examination; L13 and 14 had been removed 5 years previously	Mandible	Region of L14, edentulous, large cystic area in which was a round, radiopaque mass
35	115924	F	C	44	Hard mass protruded through membrane of mandible under lower denture	Mandible	Large, irregular, radiopaque mass, separated from the mandible by a thin translucent area
36	116894	F	C	45	Large external swelling with fluctuation	Mandible	Mottled area in edentulous mandible resembling sequestration of bone
37	111495	M	W	34	Routine roentgenologic examination	Mandible	Large radiopaque mass seemingly attached to the roots of the 3rd molar
38	110636	M	W	26	Routine roentgenologic examination (multiple cementoma)	Mandible	Radiolucent area surrounding each tooth from canine to canine, in some of which radiopaque masses were seen

TABLE II (Continued)

*Dentinoma*

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
39	134322	M	W	6	Difficulty in breathing, mucopurulent discharge for 2 years	Maxilla and maxillary sinus	Radiolucent area

*Mixed Dentinoma and Cementoma*

40	131700	F	W	26	Pain	Mandible	Large, irregular, radioopaque mass in periapical region of central necrosis
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lesion enveloping the root of a partially erupted mandibular third molar. The tumor consisted of a solid mass composed of embryonal connective tissue containing small spindle-shaped cells. No inflammatory infiltration was present. In the fibrous stage it is impossible to determine the outcome of the lesion since it may form either a dentinoma or a cementoma. Dentinoma is usual in the coronal region; cementoma, in the apical region.

The *dentinoma* in the pure form is rare; however, the same process may be found in a few of the odontogenic mixed tumors. The dentinoma, composed of odontoblastic tissue, is made up of denticles or islands of irregularly formed dentin in a stroma consisting of connective tissue with enmeshed cells of various shapes, spindle and round being more common. The dentin contains fewer and more tortuous tubules than normal and resembles secondary dentin laid down in the pulp in advance of caries. Some of the dentin encloses living cells to appear as if it had been secreted around odontoblasts. Certain of the interstitial cells closely resemble bone corpuscles and this type of tissue may be referred to as osteodentin.

The *cementoma* was described by one of us (K.H.T.),<sup>6</sup> who pointed out that it was a secondary formation, the by-product of a soft tissue tumor, the cementoblastoma (odontogenic fibroma). In the growing stage the cellular tissue is predominant, diminishing in proportion as cementum is formed. When the cellular elements have exhausted their activity, they remain as a thin connective tissue capsule around the calcified tumor; thus the histologic picture varies with the stage through which the tumor is passing. The cementoblastoma may produce cementicles which become fused, or trabeculae may be laid down

in lamellar fashion. It is difficult to distinguish cementum from bone, especially when there is cementoblastic activity as well as cementoblastic deposition. Cementum, however, has a fibrillar matrix, a more irregular lamination, and fewer inclosed cells than bone.

*Tumors Originating from Both Odontogenic Epithelium and Mesenchyma*

The odontogenic mixed tumor is composed of the epithelial and mesenchymal portions of the tooth germ with the potentialities of those structures to form enamel, dentin, cementum, pulp, and periodontal tissue. Both soft and calcified types are encountered.

*Soft Odontoma.* It has been observed that the stroma is often prominent in the solid type of ameloblastoma. Bauer<sup>7</sup> recognized that in cystic ameloblastomas there was usually an insignificant increase of the connective tissue, while in the more solid tumors the stroma was greatly increased and played an important rôle. Thoma<sup>5</sup> described soft odontoma as derived from the mesenchymal part of the tooth germ which may form from either the embryonic mesenchymal tissue or the dental follicle. He believed that soft odontoma might be classified as central fibroma or fibro-odontoma; however, as dental epithelium was present in many of these tumors, the term fibro-adamantinoma might be justified. Nagel<sup>8</sup> pointed out that these soft tumors were easily mistaken for ameloblastomas.

In odontogenic mixed tumors of the soft type, the epithelium is usually arranged in lobules or strands, the peripheral cells of which are more columnar and resemble prefunctional ameloblasts. The central cells are often squamous to spindle-shaped, or assume a stellate-reticular form, like that seen in the enamel organ. Sometimes the epithelial strands suggest buds or the bell-shape of the early enamel organ. The stroma is abundant, at times embryonal, with stellate fibroblasts as seen in the dentinal papilla and young pulp of a tooth. It may resemble more mature connective tissue in which collagen is deposited and numerous blood vessels are present. The stroma may become fibromyxomatous. Occasionally the tumor is composed almost entirely of connective tissue with epithelium a minor component.

*Soft and calcified odontomas* have been divided into two groups: the first characterized by a soft epithelial component, a soft mesenchymal component, and dentin; the second, by a soft epithelial component and enamel, soft mesenchymal component, and both dentin and cementum. The microscopic picture of the first type, as seen in case 44, was similar to that of the soft odontogenic mixed tumor except that dentin was produced. The nests of adamantine tissue were

TABLE III  
*Odontogenic Mixed Tumors*

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
41	101059	M	W	29	Painless, slow-growing, hard tumor	Mandible	Multicystic area from bicuspid to molar region and beneath the inferior border, all teeth present
42	61743	M	C	39	Swelling, pain, discharge	Mandible	Multicystic lesion involving the molar region and entire ramus
43	107940	M	W	5		Mandible	Cyst
44	118866	M	W	59	Slight swelling, paresthesia of lip	Mandible	Two large cystic lesions, broken down teeth present but apparently unrelated
45	133773	M	W	16	Swelling—central and lateral incisors tender to touch	Maxilla	Large radiopaque mass in cystic-appearing lesion, all teeth present
46	132671	F	W	35	Painless swelling	Mandible	Radiopaque masses in dentigerous cyst
47	133772	M	W	15	Gradual swelling	Mandible	Dentigerous cyst involving 2nd molar, follicular cyst in 3rd molar area, containing radiopaque masses, 3rd molar absent
48	74998	F	W	9	Failure of teeth to erupt	Mandible	Dentigerous and follicular cysts
49	95083	M	W	22	Tumor of right maxilla removed 2 years previously—fungating bleeding mass at canine fossa	Maxilla	Cystic area in canine region
50	115927	M	C	26	Slight swelling	Mandible	Irregular radiopaque mass in cystic area spreading the roots of the canine and premolar
51	63400	M	W	3	Absence of tooth—area slightly tender to percussion	Mandible	Radiopaque mass in cyst above deciduous tooth
52	127807	M	W	8	Failure of deciduous teeth to erupt	Maxilla	Small rudimentary teeth and irregular radiopaque masses
53	84173	M	W	22	Pain, thought to be due to wisdom tooth erupting	Mandible	Round, smooth, radiopaque mass rimmed by a thin line of decreased density beneath which was 3rd molar
54	121695	M	C	27		Mandible	Dentigerous cyst with odontoma in cyst, 1st molar

TABLE III (continued)

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
55	32007					Maxilla	Large, irregular, radio-paque mass in the molar region—no regular tooth formation
56	72697	M	W	21	Pain	Mandible	Cyst with radiopaque mass in 3rd molar region
57	88341	M	W			Maxillary sinus	Radiopaque mass in right maxillary sinus
58	105341	M	W	20	Asymptomatic swelling	Mandible	Large, irregular, radio-paque mass occupying the 3rd molar region and part of the ramus, displacing the 3rd molar toward the angle of the mandible
59	105338	M	W	24	Asymptomatic swelling	Mandible	Large, irregular, radio-paque mass in molar region; a molar tooth appeared beneath the mass; no teeth were present before the first premolar
60	40486	M	W	19	Pain	Maxilla	Large, irregular, radio-paque mass in 3rd molar region; the 3rd molar lay superior and anterior to the mass
61	40487					Mandible	Follicular cyst with odontoma
62	61365	F	W	18	Pain	Maxilla	Irregular, radioopaque mass in 2nd molar region—no 3rd molar evident
63	59780	M	W	25	None	Maxilla	Radiopaque mass in 2nd and 3rd molar region
64	134323	M	W	31	Irregularity in eruption of teeth	Maxilla	Irregular, radioopaque formations in region of maxillary and lateral canine—two teeth above mass

\* Army Institute of Pathology.

rimmed by dentin which was irregular in structure as is secondary dentin. The dentinal tubules were fewer and were tortuous in contrast to their regularity in normal dentin. This process could be interpreted as a response of the stroma by forming dentin in the presence of adamantine tissue, a reaction commonly observed in odontogenic mixed tumors.

Perhaps the most interesting of all the odontogenic tumors are those in which all possible phases of dental production are seen. In certain tumors, both fully formed and rudimentary teeth or irregular dental structures are found. Areas of pure adamantoblastoma, of pure dentinoma, and of epithelial tumor in conjunction with dentin production may be encountered as well as tooth buds in all stages. These processes are well illustrated in cases 46, 52, and 45. In case 46, an abortive tooth root, composed of irregularly formed dentin covered by cementum, was adjacent to small rudimentary teeth made up of an epithelial membrane around the enamel spaces. Occasional epithelial sprouts arose from this membrane. In one area the tissue consisted almost entirely of proliferating epithelium which developed in the form of irregular follicles surrounded by a rim of dentin. The epithelium consisted of an outer layer of cylindrical cells and a central portion of squamous cells which tended to form pseudo-pearls. In places, the rim of dentin was absent; in others, dentin formation was beginning. In case 52 the processes were essentially the same, but in addition there were areas of dentin laid down in denticle fashion, epithelium undergoing necrobiosis, and epithelium arranged in acini filled with a colloid-like material. In case 45, filling the cavity of a cyst was a mass of irregular dental structures in a connective tissue stroma. Between these structures were concentric masses of necrobiotic epithelial cells in which focal areas of calcification were noted. Scattered through the collections of dentin and necrobiotic epithelium were aggregates of adamantine cells, in some areas solidly packed, in others assuming a cystic pattern. In the capsule of the cyst, lobules of adamantine tissue and scattered ghost-like epithelial cells were intermingled. Of greatest interest was the formation of a small tooth bud in the capsule. It was composed of layers of epithelium, dentin, an irregular odontoblastic layer, and the dental papilla. The epithelium, however, had not differentiated into ameloblasts although a considerable layer of dentin had been laid down. These cases suggest the inductive effects of one tissue upon another. Epithelial lobules seem to evoke the potentiality of the adjacent stroma, causing dentin to be laid down. Dentin was formed despite the lack of an ameloblastic layer and in several instances in the absence of epithelium.

*Completely formed odontomas* include: (1) compound odontomas which are made up of a large number of more or less rudimentary teeth; and (2) complex odontomas in which the calcified structures bear no resemblance to the anatomic arrangement of the dental tissue. These tumors are encompassed by capsules. The pathogenesis of odontoma is brought out in case 52 (Figs. 51 to 56).

The compound type of odontoma contains a large number of teeth and is usually included in a cyst membrane. The dental epithelium, instead of forming the normal enamel organ, produces many small enamel organs which develop into tooth germs and give rise to small teeth of varying size and shape. Several adjoining tooth germs may take part in the process and produce some normal, and many deformed, teeth. Microscopically, the structure appears normal although the teeth may be irregular and dwarfed, and may be attached to one another by fibrous connective tissue, cementum, or bone.

In complex odontoma, the arrangement of the tissue is not regular and tooth-like since the tissue is in different stages of development. This tumor may be produced by a single tooth germ, which has developed abnormally, derived either from a normal tooth or a supernumerary sprout from the dental lamina. Schour, Massler, and Greep<sup>9</sup> followed the development of a complex odontoma in a group of rats in which there was hereditary anodontia of the incisor tooth. A complex odontoma is a hard, solid, rounded or oval mass, surrounded by a capsule from which it usually can be shelled out with ease. Its surface may be covered with cementum having scattered nodules of enamel. Sometimes unerupted, normally-formed teeth may be fused with the tumor. Microscopically, great variations are found in structure and in the proportion of the different tooth elements. In some, an arrangement like that of normal tooth formation is interrupted by irregularly formed tissue. In others, abnormal arrangement of tooth substance is the predominant feature, with enamel, dentin, and cementum distributed in lamellar or radial fashion. Soft tissue, such as enamel epithelium and dental pulp, may be seen between the calcified layers, the former adjoining the enamel, the latter the dentin. The cementum may be found only at the periphery beneath the epithelialized fibrous capsule.

#### INDUCTIVE EFFECTS OF ONE TISSUE ON ANOTHER

The deposition of dentin about epithelial lobules, necrobiosis of the epithelium with resultant calcification, and irregular dental patterns bring up the question of the influence of one tissue upon another. In the intimate tissue mixtures of these tumors, neighboring tissues seem to exert inductive influences upon one another, comparable to those known in embryonic development or in mixed tissue cultures. Structural features indicate that odontogenic mixed tumors are not a fortuitous jumble of independent ingredients or a potpourri of tissues, but that the form they take results from the response of one tissue to another.

An outstanding feature of the adamantoblastoma is the failure of enamel production even though certain cells are morphologically almost identical to those seen in the enamel organ of normal tooth formation. Perhaps this failure may be attributed to the fact that polarization of the neoplastic cells does not occur. It is possible that dentin influences the polarization of these cells prior to enamel production. Early dentin formation was observed in several of the soft odontogenic mixed tumors, although no enamel was laid down. Whether or not this was an intermediate stage and enamel would have formed later cannot be determined.

The relationship of the ameloblast to the odontoblast is of interest as well as of importance in the determination of the type of tissue formed and the pattern developed in odontogenic mixed tumors. Huggins, McCarroll, and Dahlberg,<sup>10</sup> by the method of transplanting developing dental tissues, found that enamel was deposited only on dentin, although dentin could be laid down independently of enamel but only when odontoblasts were present. Glasstone,<sup>11</sup> by growing portions of tooth germs *in vitro*, found that the ameloblastic layer was essential for the formation of odontoblasts. Because of these findings, Sprawson<sup>12</sup> believed that in the formation of adamantoblastomas, enamel is not formed. The cells which make up this tumor are basal cells, not ameloblasts, since odontoblasts do not become differentiated. In our study, it appeared that enamel was not formed where there was an absence of dentin and that dentin formation was independent of ameloblasts. This is shown in Figures 7, 16, and 50 and also in the two dentinomas and in like areas in the odontogenic mixed tumors. It is, however, possible that dentin production was initiated by fibroblastic activity with the resultant bone-like dentin seen in these tumors.

The dentinoma has been considered an odontoma, the predominant tissue being dentin. However, in all dentinomas previously described, enamel and cementum were present. A dentinoma consisting purely of dentin and stroma has not hitherto been reported. This tumor arises from the odontogenic fibroma which may differentiate into either cementoma or dentinoma. In one tumor studied, both tissues were present, which suggested their common origin. The structure of the dentinoma demonstrates that dentin may be produced despite the lack of odontogenic epithelium.

The stroma seen in the adamantoblastoma is easily distinguished from the connective tissue observed in the soft odontogenic mixed tumor. It is usually minimal in quantity and does not play an important rôle in the tumor. In the odontogenic mixed tumor, however,

the connective tissue is usually predominant, the epithelium scanty. In these tumors there is a tendency for the production of dentin in apposition to the epithelium. Surrounding many of the epithelial nests is a clear zone, around others a rimming of dentin of the osteodentin type with no odontoblastic layer. Like bone, the dentin has entrapped cells which appear to be of fibroblastic origin.

One must regard the tumors which arise from epithelium and connective tissue as mixed tumors. In further proof are the cases reported as adamantinosarcomas. Krompecher<sup>13</sup> reported one of the first of these. A solid, primary tumor was found in a 13-year-old boy. The histologic picture was that of spindle-cell or round-cell sarcoma, the epithelial part resembling the ordinary type of adamantoblastoma. Wigdortschink<sup>14</sup> described a similar tumor in a boy, 9 years old, who had a lesion in the mandible which appeared to be a sharply circumscribed cyst. Besides highly differentiated adamantine tissue, it contained very cellular connective tissue of a fibrosarcomatous character.

The odontogenic mixed tumor may arise as either a soft or a calcified tumor, the epithelial and mesenchymal elements varying as to differentiation and proportionate quantity. The soft odontogenic mixed tumor is composed of epithelial and mesenchymal elements, and in those observed the connective tissue predominated. Morphologically, it resembles the dental pulp, the cells being stellate. The hard, calcified, odontogenic mixed tumor has been frequently described in the literature and has been termed odontoma. The frequent occurrence of soft epithelial polyhedral and squamous cells as well as adenomatous structures in this hard tumor was noted; in many instances these cells were of adamantine nature like the cells of pure adamantoblastoma. Occasionally, irregular calcospherites were seen in the epithelial nests.

The combination of soft and calcified odontogenic mixed tumor produces many bizarre pictures, because of the varying proportions and arrangements of the epithelial and mesodermal elements, resulting from splitting, budding, and fusion of the odontoblasts.

#### LIMITATION OF GROWTH

From the study of this group of tumors, it appears that differentiation to a variety of forms may take place, depending to a large extent on the development of the epithelium and mesoderm at the time the neoplasm begins to form. It seems that the uncalcified tumors undergo a quantitative growth with but little qualitative alteration, while the calcified ones undergo a quantitative and a qualitative change, that is, their growth is one with differentiation. Where evolution is chiefly quantitative, the possibility of uncontrolled growth is far greater than

when the evolution is chiefly qualitative, for differentiation tends to lead to the development of the final form. Thus we find that the adamantoblastoma is inclined to grow large and be invasive, while the odontoma appears to grow only to a limited size. The odontogenic mixed tumor has the developmental potentialities of both adamantoblastoma and odontoma, a fact that has received further proof from this series of cases.

#### SUMMARY

The odontogenic tumors are classified into three groups: epithelial, mesenchymal, and mixed. The dentinoma, a pure mesenchymal tumor, is composed of connective tissue in which denticles or islands of irregularly formed dentin are present. The odontogenic mixed tumors consist of epithelial and mesodermal elements which are in combination in various proportions and arrangements. Three types are recognized: soft, soft and calcified, and calcified. The soft type has been differentiated from the solid adamantoblastoma.

There is evidence of the inductive influences of one tissue on another in the odontogenic mixed tumors. It is noted that epithelium in these tumors seems to stimulate dentin formation, but that the presence of epithelium is not necessary for the production of dentin. Also, dentin is formed in the presence of epithelial cells not differentiated into ameloblasts. Neoplastic adamantine tissue and enamel-forming ameloblasts have been distinguished. The presence of these two types accounts, in part, for the formation of the soft and calcified odontogenic mixed tumors.

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#### DESCRIPTION OF PLATES

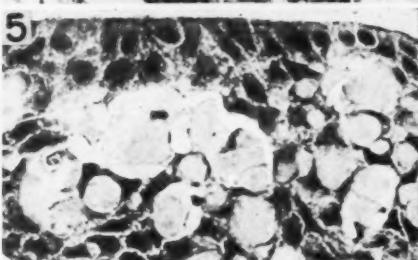
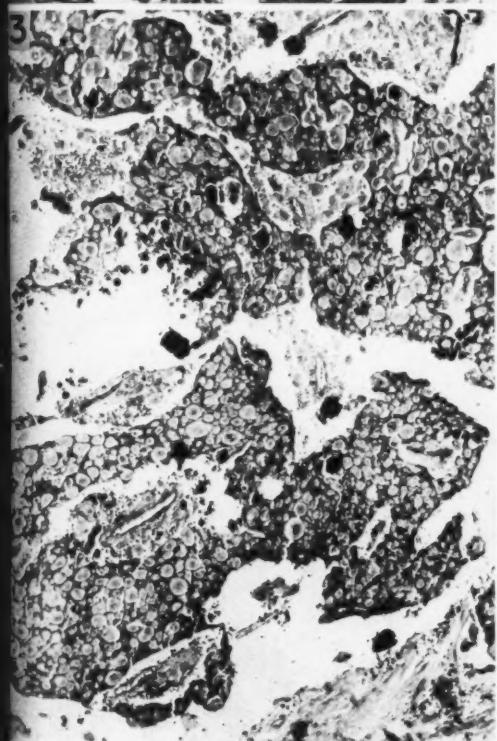
##### PLATE 91

FIG. 1. Case 24. *Adamantoblastoma*. A.I.P. Acc. 65091, Neg. 85499. A growth which had caused no pain, was removed from the maxillary region of a woman, 60 years of age. The tumor was made up of sheets of large epithelial cells. Around the edges these cells were palisaded as in the enamel organ (Text-Fig. 1). The central portion consisted, for the most part, of squamous cells, with stellate reticulum of enamel organ type in a few areas. The tumor was almost purely epithelial, with little fibrous stroma evident.  $\times 60$ .

FIGS. 2 to 5. Case 25. *Adamantoblastoma, adenoid type*. A.I.P. Acc. 118679. A man, 32 years of age, had noted progressive painless swelling on the side of his face for 9 years. Roentgenograms showed a large cystic lesion enveloping the crown of the cuspid tooth (Fig. 2, Neg. 83938). The lesion was removed. Surrounding the root of the cuspid tooth was an overgrowth of brownish gray, relatively soft tissue with numerous, tiny, hard masses loosely grouped together, and other discrete nodules. Microscopically, a rather acellular fibrous tissue stroma surrounded masses of epithelial cells which, for the most part, were polyhedral with prominent intercellular bridges (Fig. 3, Neg. 84047,  $\times 70$ , and Fig. 4, Neg. 84051,  $\times 335$ ). In some instances the cells were oval to columnar and tended to stain lightly, resembling ameloblasts in nuclear detail, position of the nucleus, and intercellular substance (Fig. 5, Neg. 87683,  $\times 450$ ). The nuclei were oval or slightly elongated and of uniform size. The cells were arranged in acini containing a mucoid substance in which there was evidence of focal calcification. Occasionally the cells themselves were calcified, in some areas so heavily that the underlying epithelium was masked.







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Figs. 6 and 7. Case 39. *Dentinoma*. A.I.P. Acc. 134322.\* The left nostril of a 6-year-old boy was completely obstructed by a firm, moveable mass, which had produced slight asymmetry of the face. Deviation of the septum to the right caused difficulty in breathing through the right nostril. The bone over the maxillary sinus could easily be depressed over an area about an inch in diameter. The first dentition was complete. A rather loose mesenchyma contained numerous round and irregular masses of eosinophilic material recognized as dentin (Fig. 6, Neg. 85488,  $\times 90$ ). This substance was somewhat fibrillar with irregular dentinal tubules; entrapped cells were common (Fig. 7, Neg. 85489,  $\times 370$ ). One part of the tumor was composed exclusively of connective tissue which resembled dental pulp; at the border of the tumor the tissue contained bony spicules which corresponded to the normal bone surrounding the tumor.

Figs. 8 to 11. Case 41. *Soft odontogenic mixed tumor*. A.I.P. Acc. 101059. A painless mass on the face of a man, 29 years old, was firm except for one fluctuant area anterior to the angle of the mandible. Roentgenologic examination revealed a multicystic area (Fig. 8, Neg. 78852). A large neoplasm had invaded much of the substance of the mandible. The larger portion of the tumor was on the mesial side of the bone, but there were two solid masses within the body of the mandible. The lower border was completely eroded and replaced by a tumor with a bluish surface. It cut with relative ease, was compact, light-yellow, gelatinous, interspersed with a few fine trabeculae, and was surrounded by a thick fibrous capsule (Fig. 9, Neg. 81565). Microscopically, large columns and strands of epithelial cells were embedded in a fibrous matrix. Many epithelial strands were thin and multibranching, winding through contiguous microscopic fields (Fig. 10, Neg. 78576,  $\times 85$ ). In the individual cord the cells in the outermost layer were columnar; those in the center were either squamous or small with clear cytoplasm, giving the tissue a reticulated appearance. Connective tissue of a simple fibrous type was far more abundant in the tumor than epithelial tissue. In many areas, the cells of the stroma were irregularly disposed, their cytoplasm clear, and the nuclei large with prominent nucleoli (Fig. 11, Neg. 85502,  $\times 405$ ).

\* Case sent to K.H.T. by Dr. Louis Berger, Montreal, Canada.





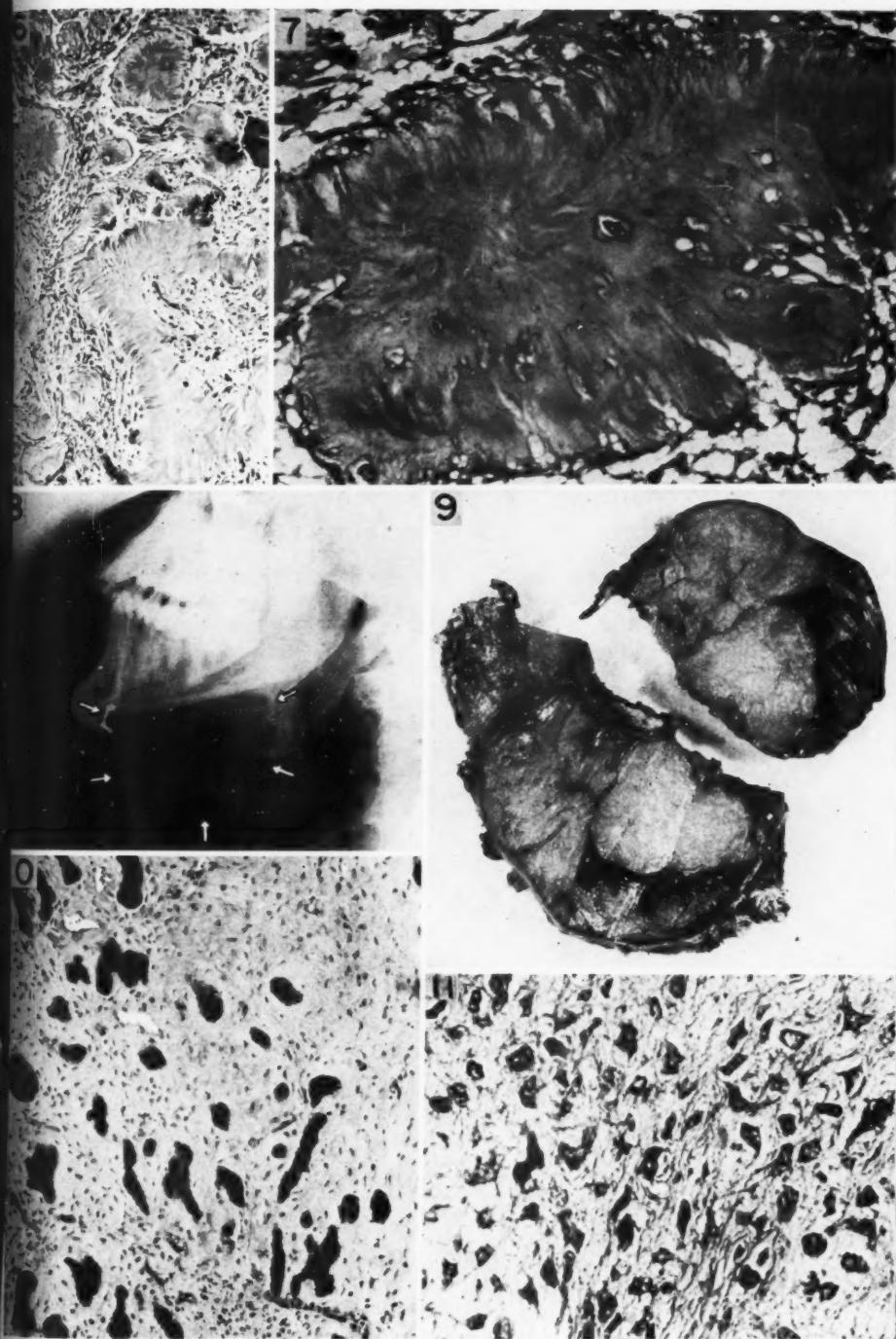


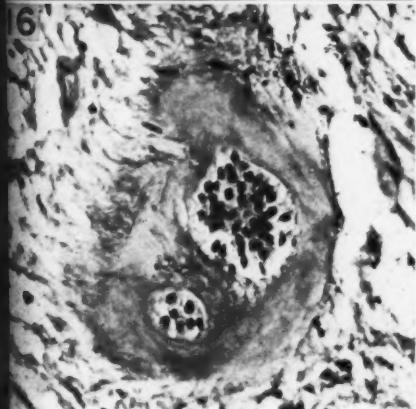
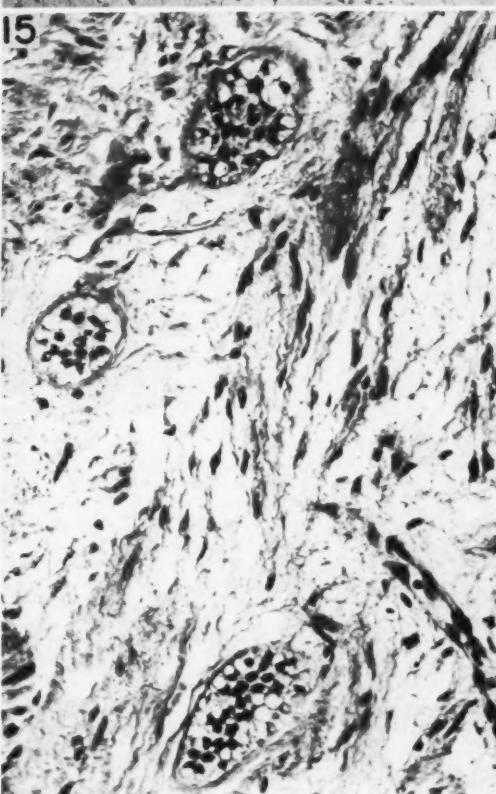
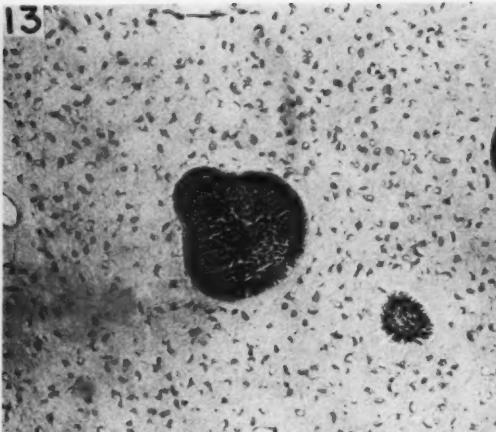
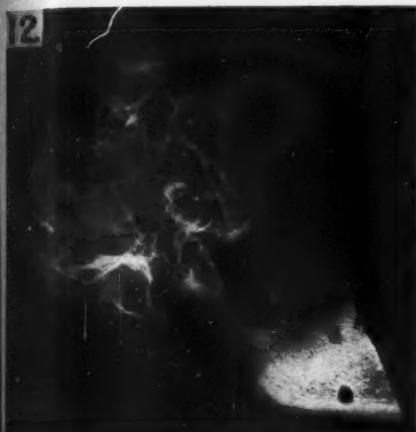
PLATE 93

FIGS. 12 and 13. Case 42. *Soft odontogenic mixed tumor*, A.I.P. Acc. 61743. Four years previously a swelling had appeared on the left side of the face of a man, 39 years of age. Two years later limitation of motion of the jaw became apparent. A hard mass extended from the border of the mandible to the temporal region. Roentgenologic examination revealed a large multicystic mass the size of an orange and an unerupted tooth (Fig. 12, Neg. 68186). The left ramus and body of the mandible up to and including the second molar were resected. In several areas the tissue was composed of occasional epithelial strands and lobules in a cellular and edematous connective tissue. The peripheral cells of the lobules were inclined to be columnar while the central cells were squamous and many had assumed the appearance of stellate reticulum (Fig. 13, Neg. 85498,  $\times 100$ ). Several areas of the connective tissue portion of the tumor resembled the connective tissue of the dental papilla of tooth formation.

FIGS. 14 to 16. Case 44. *Soft odontogenic mixed tumor with production of dentin*. A.I.P. Acc. 118866. A man, 59 years old, had first observed a slight swelling on the labial aspect of the incisor region 15 years previously. It gradually grew larger. Roentgenograms disclosed two large radiolucent areas in the anterior right portion of the mandible, each associated with a small root (Fig. 14, Neg. 82707). Microscopically, interlacing dense bands of collagen fibers were loosely packed and scattered throughout the loose stroma. Lobules and strands of small, deeply stained cuboidal cells with clear cytoplasm suggested pre-ameloblasts (Fig. 15, Neg. 85500,  $\times 275$ ). Surrounding some of the lobules were rims of eosinophilic material which was identified as dentin (Fig. 16, Neg. 85495,  $\times 305$ ).







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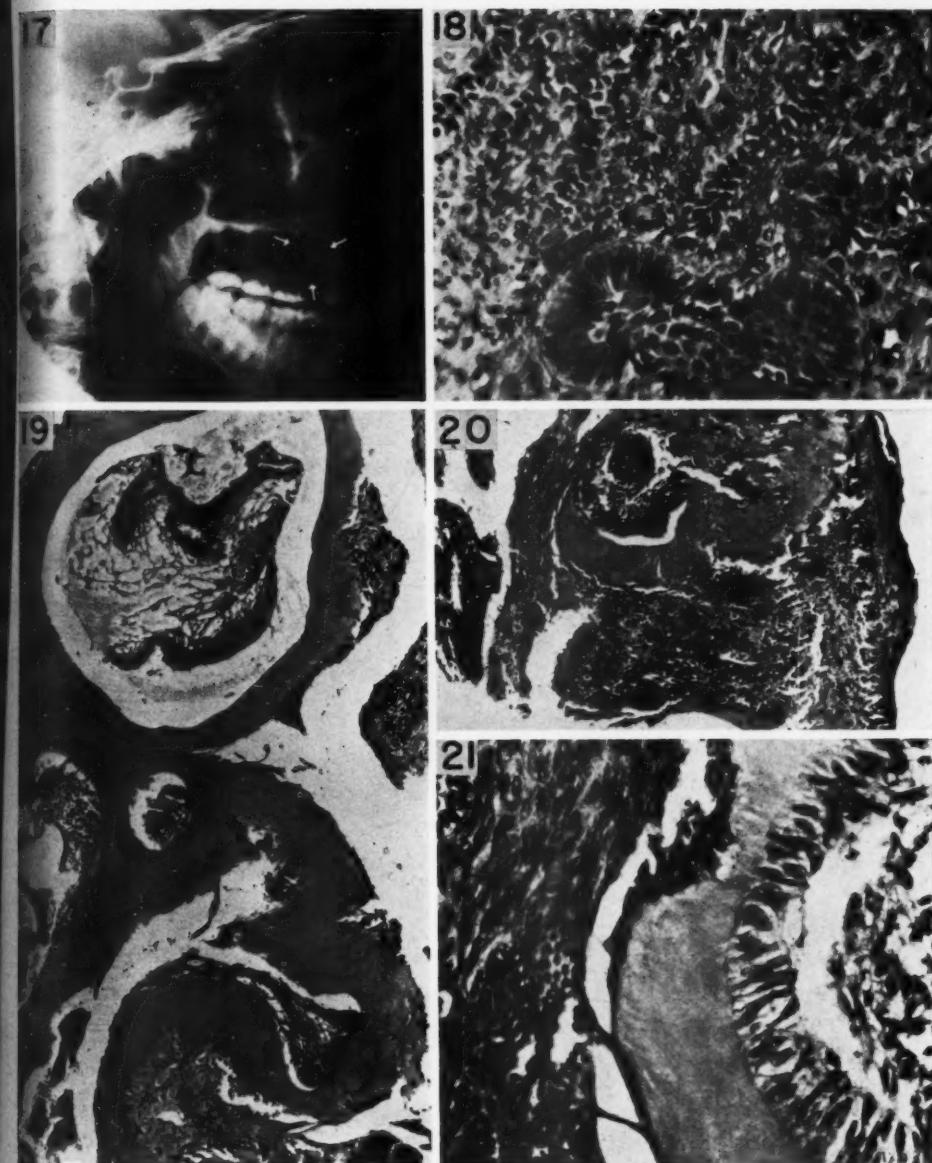
Odontogenic Tumors

PLATE 94

FIGS. 17 to 21. Case 49. *Odontogenic mixed tumor*. A.I.P. Acc. 95083. Following operation for a tumor of the right maxilla 2 years before, the gum of a man, 22 years old, had gradually increased in size. There was a fungating, bleeding mass in the right canine fossa. Roentgenologic examination disclosed a cystic lesion in the canine region (Fig. 17, Neg. 82113). Fragments of the tumor were hard. The sections varied from proliferating adamantine epithelium (Fig. 18, Neg. 82265,  $\times 270$ ) to abortive dental formations (Fig. 19, Neg. 82264,  $\times 75$ ). Numerous fasciculi of spindle cells were fringed with columnar cells, and in some areas the columnar cells formed radial collars about the spindle cells. One section consisted of masses of adamantine epithelium surrounded by irregular formations of dentin like those seen in the other odontogenic mixed tumors. Rimming the dentin were small, round, deeply staining cells which did not have the structure of odontoblasts, but, nevertheless, seemed to be the cells responsible for the formation of the dentin (Fig. 19, and Fig. 20, Neg. 82263,  $\times 105$ ). Focal calcification of occasional nests of epithelial cells was found. Lining the inner surface of many of the dentin masses were columnar cells morphologically like ameloblasts; the central cells were stellate (Fig. 21, Neg. 82262,  $\times 95$ ).







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FIGS. 22 and 23. Case 51. *Odontogenic mixed tumor*. A.I.P. Acc. 63400. A white boy, 3 years of age, had a swelling over the gum in the lower left deciduous cuspid area where there was no tooth. Roentgenologic examination revealed a cystic lesion which contained small coalesced calcified bodies (Fig. 22, Neg. 78794). The permanent canine could be seen beneath the cystic area. Microscopically, a very loosely formed tissue was composed of stellate and spindle cells such as are seen in the stellate connective tissue of the pulp. Partially covering the section were epithelial cells of columnar type with large, darkly staining nuclei, which appeared like those found in the inner enamel epithelium. Lying in the connective tissue of embryonal type were lobules of epithelial cells (Fig. 23, Neg. 78584,  $\times 360$ ). The calcified bodies were not examined microscopically.

FIGS. 24 to 27. Case 48. *Odontogenic mixed tumor*. A.I.P. Acc. 74998. Delayed eruption of the lower right molar was noted in a girl, 9 years of age. The roentgenogram revealed the crown of an embedded first molar projecting into a cyst. The second and third molars were absent and this area was occupied by a large cystic lesion (Fig. 24, Neg. 78798). The mass from the second molar region consisted of rather dense, moderately cellular stroma, most of the cells being ovoid or stellate. Interspersed were islets of adamantine epithelial cells, arranged in cords and strands (Fig. 25, Neg. 78580,  $\times 105$ ), some resembling tooth buds (Fig. 26, Neg. 78683,  $\times 70$ ). Some of these islets showed early central cystic degeneration. In one area, an attempt at tooth formation was seen; an inner enamel layer and stellate reticulum could be recognized (Fig. 26); adjacent to this area, a calcified, irregularly formed tissue was being laid down. Several masses of dense hyaline material showed early calcification (Fig. 27, Neg. 78684,  $\times 70$ ). A portion of the tumor, in which there were lobules of epithelial cells, resembled adamantoblastoma.





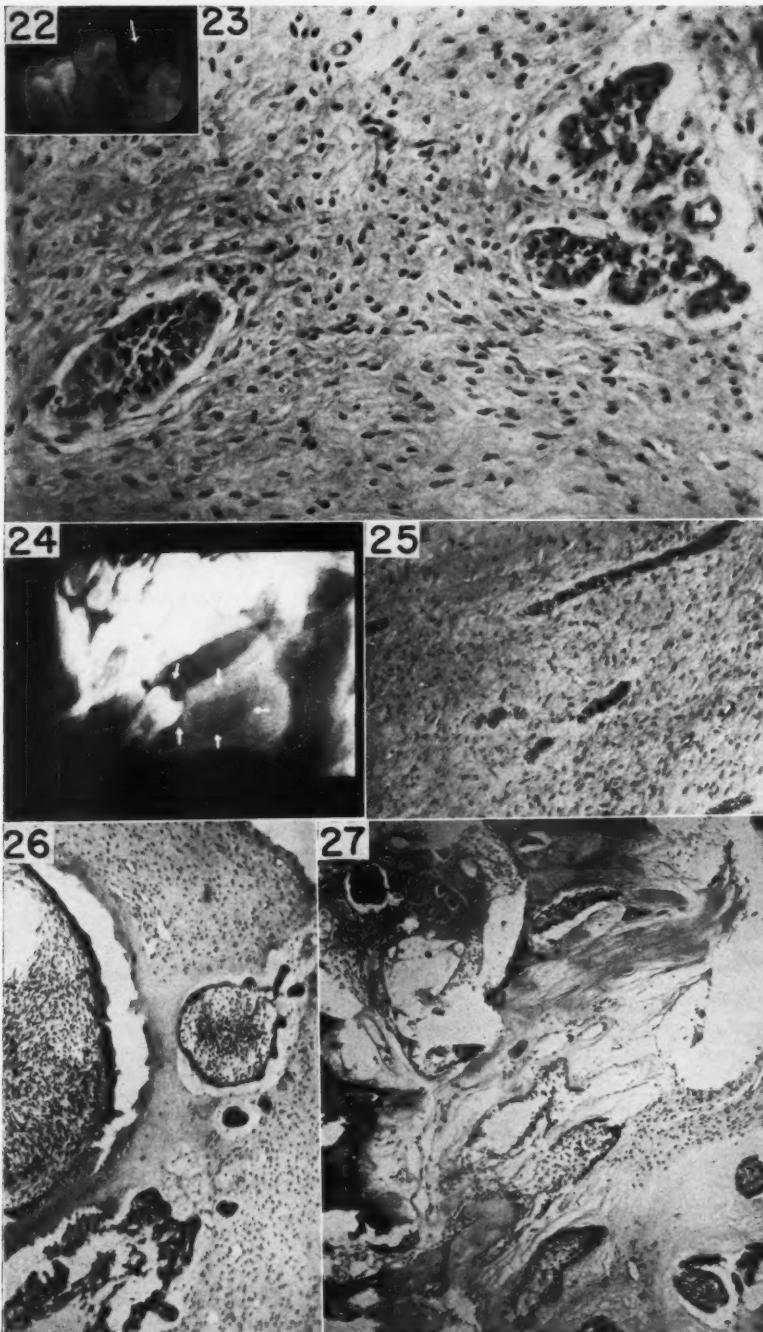
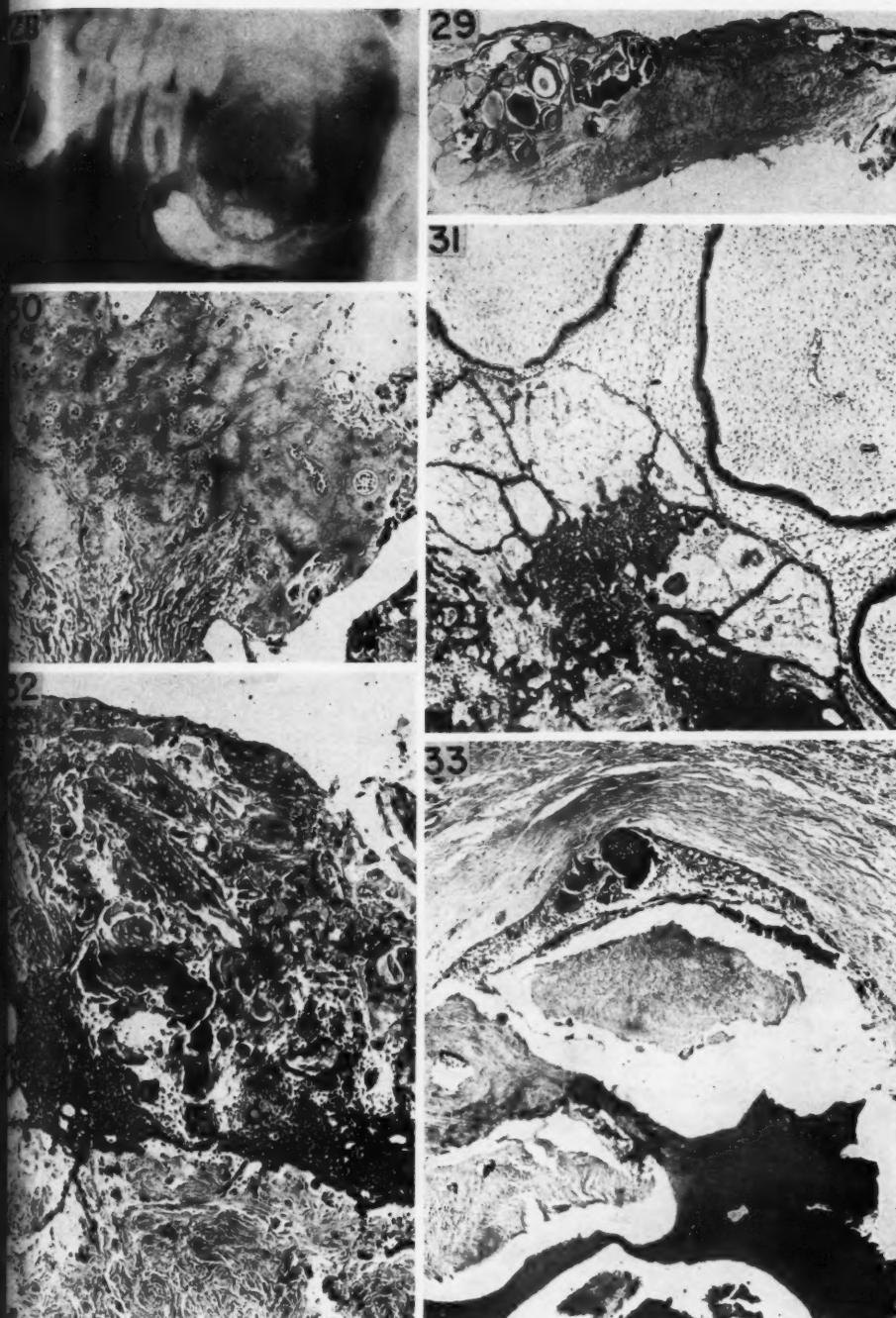


PLATE 96

FIGS. 28 to 33. Case 47. *Odontogenic mixed tumor*. A.I.P. Acc. 133772. A swelling on the right side of the face of a boy, 15 years old, was confined to the ascending ramus. Gradual increase in size and slight pain noticeable for 9 months were attributed to a blow. On the right side of the mandible the second and third molars were missing. The roentgenogram showed a two-compartment cyst: the first, below the roots of the first molar, contained a tooth displaced to the inferior border; the second, which occupied the entire ramus and expanded its anterior border, contained a mass of round calcified particles (Fig. 28). Microscopically, the cyst capsule (Fig. 29, Neg. 85478,  $\times 4$ ) was composed of dense connective tissue lined by adamantine epithelium, some of which was necrobiotic (Fig. 32, Neg. 85491,  $\times 90$ ). The adamantine cells were, for the most part, packed closely and their nuclei stained deeply. Attached to the adamantine tissue and situated in the capsule were many embryonal tooth organs composed of a layer of ameloblasts surrounding a dental papilla (Fig. 31, Neg. 85494,  $\times 90$ ). An attempt to form an odontoblastic layer was noted. Between the tooth organs was a stellate reticular tissue (Fig. 31), and in some of them enamel and dentin were formed (Fig. 29). Attached to the functional ameloblastic layer was neoplastic adamantine tissue (Fig. 31), the cells of which tended to be arranged around small cysts. In several places in the capsule, masses of irregularly formed dentin with sparse and irregular tubules were laid down; cellular inclusions were found in the dentin (Fig. 30, Neg. 85492,  $\times 100$ ). In one area there was an irregular formation of tooth substance as in a complex odontoma (Fig. 33, Neg. 85493,  $\times 60$ ).







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PLATE 97

Figs. 34 to 40. Case 46. *Odontogenic mixed tumor*. A.I.P. Acc. 132671. A white woman, 35 years old, had had a painless swelling of the left side of the face for 1½ years (Fig. 34, Neg. 86219). A firm mass over the left ramus of the mandible was shown by roentgenologic examination to contain a large cystic cavity involving the entire left ramus and part of the mandible, and to encase the crown of a molar tooth. Above the tooth were numerous, fused, radioopaque masses (Fig. 35, Neg. 86210). The material removed at operation consisted of an irregular, reddish pink, smooth sheet of membranous tissue and attached fibrous tissue in which several firm, white, smooth, encapsulated nodules were embedded. At one corner of the sheet was an irregular reddish pink, mucosa-covered mass of firm tissue studded with many pearly white, round, smooth calcified nodules. In the center was an abortive tooth. Microscopically, the large calcified mass (described as the abortive tooth root) was made up of irregularly formed dentin and covered by projections of cementum; close by were small rudimentary teeth (Fig. 36, Neg. 85480,  $\times 4$ ). Epithelial membranes surrounded spaces which represented the enamel dissolved during decalcification. Some of the more immature enamel was still attached to a core of dentin which in some instances contained a central pulp canal. Connective tissue surrounded some of the epithelial membrane, and, occasionally, cementum extended from the tooth to join the connective tissue. Lining a cystic space and extending into the connective tissue were masses of small, deeply staining epithelioid cells which had a tendency to form small acini (Fig. 37, Neg. 85501,  $\times 95$ ). At one place, epithelial structures, rimmed by dentinoid, had proliferated into the adjoining tissue (Fig. 39, Neg. 85483,  $\times 100$ ); at another, proliferation of epithelium had produced irregular papillary structures surrounded by connective tissue. The papilla was made up of an outer layer of cylindrical cells and contained a variety of cells, some of which had undergone squamous metaplasia and had formed pseudo-pearls. Rimming many of these epithelial structures was an eosinophilic material which consisted of irregular tubules and a few cellular inclusions in a somewhat fibrillar material. This material was identified as dentin (Fig. 38, Neg. 85505,  $\times 100$ ). Scattered through the connective tissue capsule were lobules of adamantine cells, many groups of which showed central cystic degeneration (Fig. 40, Neg. 86945,  $\times 160$ ).





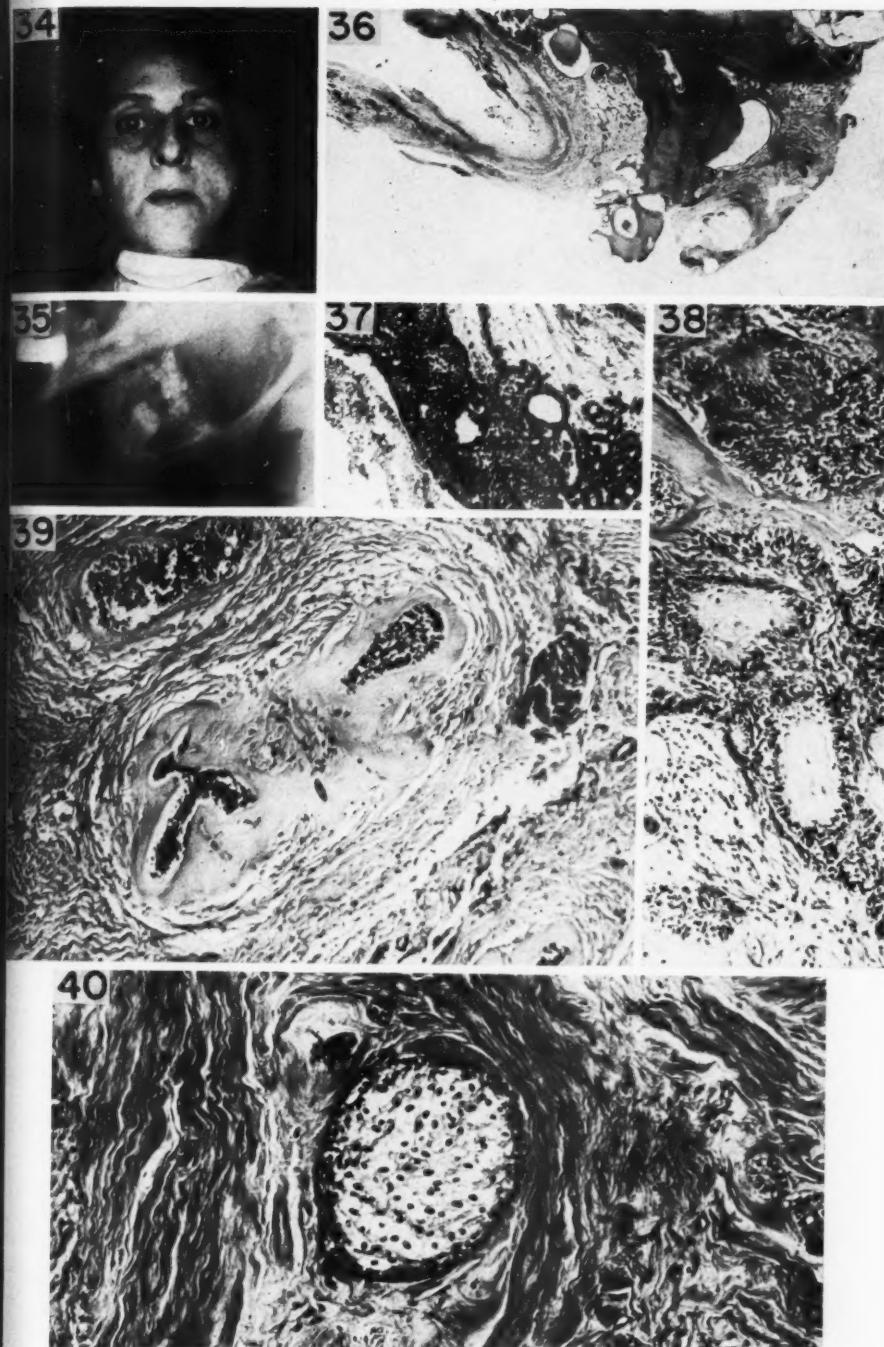


PLATE 98

FIGS. 41 to 45. Case 50. *Soft and calcified odontogenic mixed tumor.* A.I.P. Acc. 115927. A slight swelling had appeared 3 days before in the premolar region of a man, 26 years of age. Roentgenologic examination revealed an irregular radiopaque area in the premolar region (Fig. 41, Neg. 82117). Some of the tissue removed at operation was hard as bone; some was soft and fibrous. The sections showed a complex arrangement of tooth elements surrounded by areas of degenerated epithelium (Fig. 42, Neg. 85497,  $\times 6$ ). The dental structures were irregularly formed, entwined, and sometimes attached by a light-staining, eosinophilic, bone-like substance of the character of osteocementum. The aborted teeth were covered by varying amounts of enamel, the matrix being retained and not entirely removed during decalcification. Cementum covered the coronal portions of the teeth. Attached to the osteocementum and occupying the remaining portions of the specimen were ghost-like epithelial cells (Fig. 43, Neg. 85486,  $\times 315$ ). Situated between the irregular dental structures were masses of epithelial cells, polyhedral or flattened, having a tendency to arrange themselves around small spaces; in some, focal calcification was noted (Fig. 44, Neg. 85487,  $\times 315$ ). In one section which consisted of the capsule of the cyst, the lining was composed of squamous epithelium with spindle-shaped nuclei and epithelium in the acinar arrangement previously described. Islands of adamantine tissue were evident in the fibrous connective tissue capsule and in one area was seen a ring of dentin, its outer aspect covered on one side by adamantine epithelium, its center consisting of long fibers with relatively few cells. Calcification was seen in one area. The dentin contained irregular tubules and an occasional entrapped cell (Fig. 45, Neg. 85504,  $\times 185$ ).





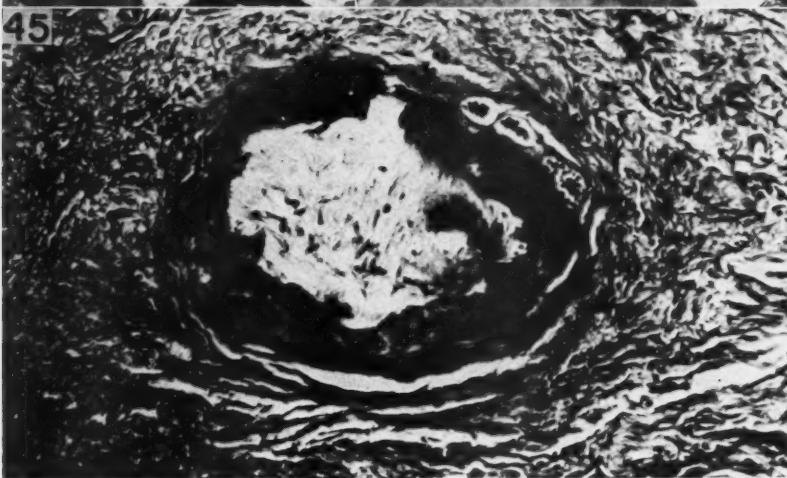
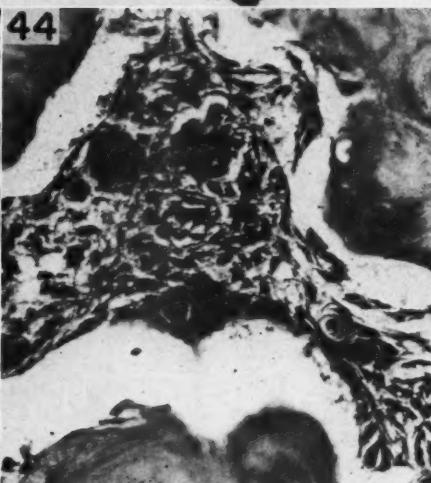
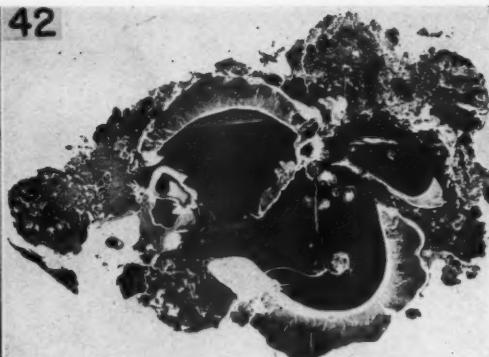
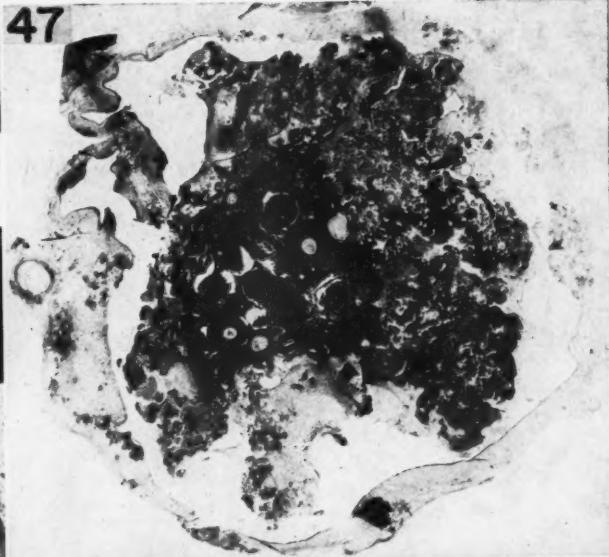


PLATE 99

Figs. 46 to 50. Case 45. *Odontogenic mixed tumor*. A.I.P. Acc. 133773. A boy, 16 years of age, noticed a swelling under his upper lip which had been growing for several months, and tenderness of the left maxillary lateral and central incisors. Bulging of the labial surface of the alveolar process prevented the lips from closing. The swelling was firm but crepitant. The roentgenogram disclosed a cystic area extending from the alveolar process of the anterior part of the maxilla into the palate. In the center was a radiopaque mass, irregular and unevenly calcified (Fig. 46). Microscopically, the cyst was composed of vascular fibrous connective tissue lined by stratified squamous epithelium. Springing from one side of the cyst wall and nearly filling the cavity was a mass of tooth-like material in connective tissue stroma (Fig. 47, Neg. 85497,  $\times 7$ ). Two or three of the structures were nearly normal teeth on a small scale, the others were smaller, less well differentiated, and without pulp centers. Although the dentin was laid down normally, the enamel was not arranged correctly. Necrobiotic epithelial cells arranged in concentric masses, resembling the spider cells of an adamantoblastoma, were found largely between the small, poorly differentiated teeth (Fig. 48, Neg. 87684,  $\times 70$ ). In some of the masses, focal areas of calcification were seen. Aggregations of adamantine epithelial cells were scattered among collections of dentin and epithelial ghost cells. Most of this adamantine tissue was solidly packed but in some areas it assumed a cystic pattern, the spaces being filled with a colloid-like material. Here and there dentin had been laid down haphazardly. In the capsule were accumulations of the same necrobiotic epithelial cells as in the lumen, also lobules of epithelial cells of distinctly adamantine nature. The peripheral cells were either cuboidal or columnar, resembling the prefunctional ameloblast (Fig. 49, Neg. 85503,  $\times 430$ ). Of greatest interest was the formation of a small tooth bud in the cyst capsule. Structurally it was composed of epithelium, dentin, and dental papilla, but the epithelium did not suggest functional ameloblasts and there was no attempt at enamel formation. A considerable layer of dentin was evident, although the odontoblastic layer was not regular or even fully developed; the dental papilla was characteristic (Fig. 50, Neg. 85484,  $\times 85$ ).







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Odontogenic Tumors

PLATE 100

FIGS. 51 to 56. Case 52. *Odontogenic mixed tumor*. A.I.P. Acc. 127807. A boy, 8 years old, had a diastema between the maxillary central incisors and a hypertrophied labial frenum. On the right were the two permanent incisors, on the left the two retained deciduous ones. Roentgenologic examination disclosed a calcified mass (Fig. 52, Neg. 85191), distal to which were three unerupted teeth. Six years before, the left central deciduous incisor failed to erupt normally and a small cystic lesion was noted in the roentgenograms but was not removed (Fig. 51, Neg. 85191). This history suggested an odontoma which had formed in the cystic area and which probably had at first been soft but later had expanded to form calcified dental structures. Microscopic examination showed that the mass was composed of the various elements of the tooth, arranged in the irregular pattern consistent with odontoma (Fig. 53, Neg. 85556,  $\times 6$ ), and was encapsulated by fragmentary connective tissue which in one area contained adamantine epithelium arranged in acini filled with a mucoid substance. Lying in dentin or enamel spaces were collections of ghost epithelial cells which had undergone calcification (Fig. 54, Neg. 85553,  $\times 180$ ). In some areas there was evidence of necrobiosis of the epithelium preceding calcification. Attached to the normal dentin were masses of irregularly formed osteodentin around ghost-like epithelial cells (Fig. 55, Neg. 85555,  $\times 120$ ). At one place remnants of an ameloblastic layer lay over an enamel space; this layer was adjacent to flattened epithelium which inclined toward an acinar arrangement (Fig. 56, Neg. 85558,  $\times 480$ ).





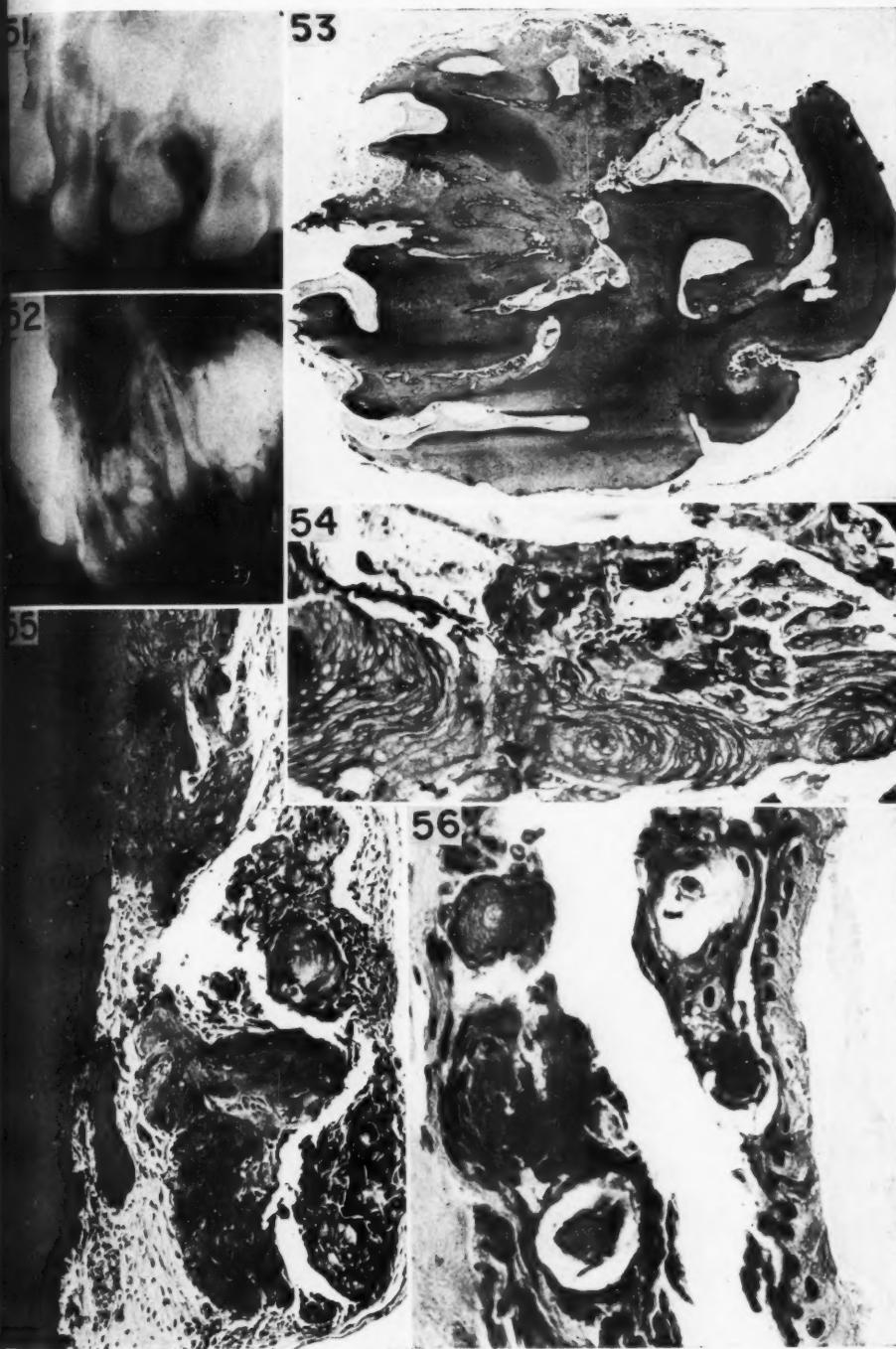


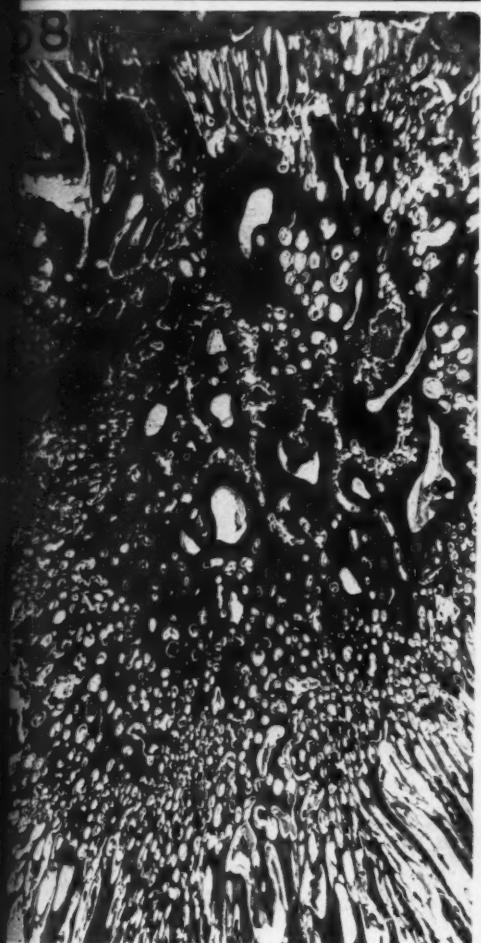
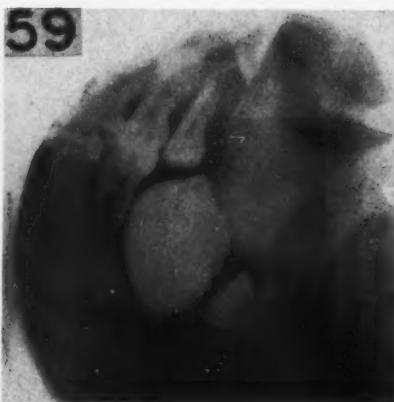
PLATE 101

FIGS. 57 and 58. Case 59. *Odontogenic mixed tumor*. A.I.P. Acc. 105338. A man, 24 years of age, had been aware of an asymptomatic swelling of the face for about 1 year. There was an edentulous area distal to the first premolar. The roentgenogram revealed a large calcified mass, beneath which there was a molar tooth. An osteolytic area suggested that the mass was lying in a cyst (Fig. 57, Neg. 78297). Microscopically, a diffusely pink substance contained numerous excavations which made it appear cancellous (Fig. 58, Neg. 78793,  $\times 8$ ). For the most part this substance was dentin; adjacent, and lying free in some of the spaces, was cementum. Cementicles were present, especially in areas which tended to simulate periodontal membrane. There was a tendency to form regular tooth structure; however, this differentiation was not achieved. The numerous excavations were enamel spaces. Partially surrounding the mass was a narrow zone of granulation tissue and beyond this, one of dense young connective tissue.

FIGS. 59 and 60. Case 58. *Odontogenic mixed tumor*. A swelling of the right side of the face of a man, 20 years of age, was asymptomatic and of a few years' duration. Roentgenologic examination revealed a large calcified mass, consistent with odontoma, extending from the second molar region back to the ramus, and down almost to the border of the mandible. Lying posterior to this mass was a cystic area into which the crown of an impacted third molar protruded (Fig. 59, Neg. 78795). In sections, spicules of dentin arranged in a heavy network were lined in some areas by cementum. Between the spicules there were oval, elongated, usually empty channels, some of which, however, contained remnants of enamel. There were spaces where the enamel had been dissolved by decalcification. At one edge, an embedded nodule resembled a portion of a distorted tooth with a dentin core, an enamel cap, a pulp chamber with a regular odontoblastic layer, and a few denticles lying in the pulp (Fig. 60, Neg. 78796,  $\times 6$ ). The attached capsule was composed of connective tissue in which strands, lobules, and cords of adamantine epithelial cells were present. In one area a cystic structure was seen.

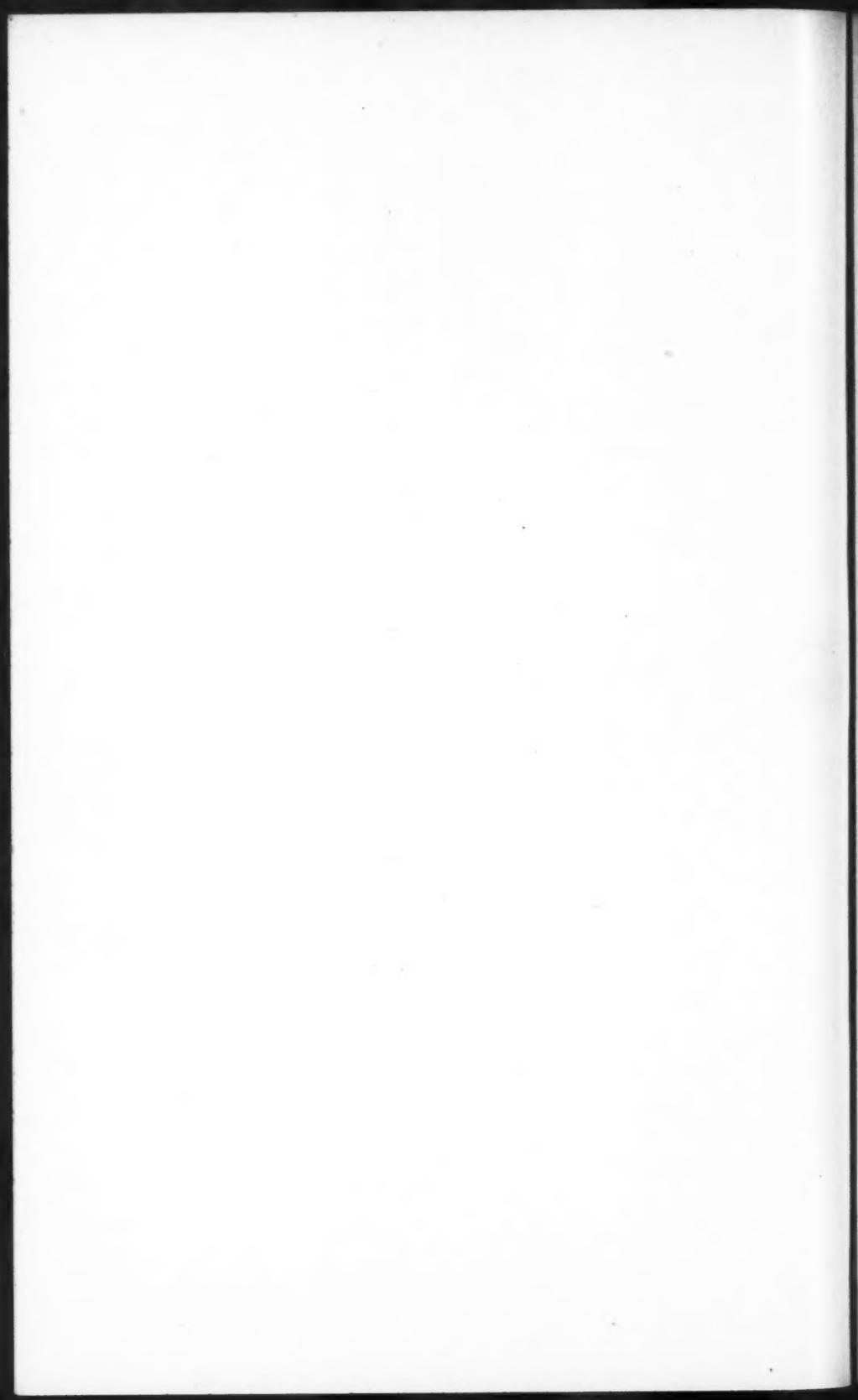






Thoma and Goldman

Odontogenic Tumors



## ATYPICAL LICHEN PLANUS \*

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(From the Schick General Hospital, Clinton, Iowa)

### INTRODUCTION

I have had opportunity to examine a number of specimens taken for biopsy from patients who were being treated for a skin disease which had its origin in the Southwest Pacific area and which, for lack of a better designation, has been called atypical lichen planus, or lichenoid dermatitis. Since beginning this study I have also been able to examine one patient who presented similar clinical and histologic findings, but whose disease began in Italy. Clinically, all these cases showed various manifestations: papular, eczematoid, nodular, or hypertrophic. Pathologically, they also presented a variety of features depending on their duration or severity. In all, 21 specimens were examined.

Although the cases cannot be divided into precise categories because the disease has a gradual evolution from acute involvement to final healing, nevertheless, on a morphologic basis, it has been possible to classify the microscopic picture roughly into three stages. These have been called (1) acute, (2) subacute, and (3) chronic or healing.

Histologically, these stages were seen to merge with one another so that occasionally the central part of a section would indicate one stage of the disease while the peripheral portion would have a different appearance. Nevertheless, the classification is of some value, for besides indicating the severity of the process in the sections examined, it also calls attention to the fact that more than one histologic pattern can be expected in a biopsy examination. Of the 21 specimens examined, 6 were considered as essentially acute, 7 as essentially subacute, and 8 as chronic.

To illustrate these three gradations, three fairly typical examples have been chosen.

### ILLUSTRATIVE CASES

#### Case 1 (Acute Stage)

This patient was a staff sergeant in a baking company. He left the United States on September 21, 1943, and arrived in Brisbane, Australia, on October 23, 1943. He remained there 6 weeks and then left for New Guinea. He arrived in Milne Bay on December 1, 1943. He remained there for 2 months and then went to Oro Bay, where he arrived on February 10, 1944.

*Diet.* While he was stationed in Australia, he had a very adequate diet, including fresh meat, vegetables, and fruits each day. His diet in New Guinea, he stated,

\* Received for publication, October 27, 1945.

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seemed quite adequate. He had fresh meat four or five times a week and fresh vegetables and fruit quite often.

*Atabrine.* Atabrine was started on November 20, 1943. The dose was 0.1 gm. daily until December 1, 1943, when it was increased to 0.2 gm. daily. About three times a month he would take six tablets a day for 3 days. These were periods after he had been in the jungle and had been bitten by mosquitoes. He had no symptoms to suggest atabrine sensitivity.

*Tropical Diseases.* He had no malaria, dengue, scrub typhus, or tropical ulcer. On one occasion he was bitten by many small red ants but knows of no other insect bites.

*Geographical Location.* He was stationed at Milne Bay and Oro Bay. His campsite at Oro Bay was located in an area that had already been cleared. It was near swampy land where there were many trees. He went into the jungle on several occasions to cut and peel trees. During these periods he was exposed to vegetation to a rather marked degree. He stated that natives came to work in their camp area quite frequently, but at no time did he get any closer to them than 3 feet.

His skin trouble started on March 1, 1944, when, following peeling of small trees and getting some of the sap on his wrists, he noticed itching at those regions. About 10 days later he developed a fine vesicular eruption over the dorsum of both hands. He entered the hospital on April 22, 1944, with a mild fever and a rather marked edema over the dorsal surface of both hands. These regions were quite tender. He was transferred to a general hospital where examination on April 27, 1944, revealed a generalized, lichenoid, papular eruption with bullous lesions on the lips and buccal mucous membrane. He was evacuated to the zone of the interior with his skin condition improved, but he noticed an increase of pigmentation.

*Physical Examination.* Examination revealed an undernourished soldier who appeared older than the stated age. There was a heavy atabrine pigmentation of the skin. There was a markedly erythematous, generalized, papular and nodular eruption which was accentuated on the extensor surfaces of the elbows and knees, as well as over the sacrum and the heels. Numerous lesions of the same type were present on the scalp, especially on the posterior portion where there were areas of patchy hair loss. There were many small, milky white papules on the mucous membranes of the cheeks. The rest of the general physical examination was negative.

#### Microscopic Description

Sections (Fig. 1) showed a central area with marked cellular infiltration and peripheral regions of more chronic inflammation.

The stratum corneum was variously thickened. In some areas this thickening was prominent; in others it appeared only as a few strands of horny tissue. The openings of the hair follicles were occasionally seen to be dilated and to be filled with masses of keratin, some of which contained the remnants of nuclear material. No definite parakeratosis was seen. The granular layer also appeared variously involved. In some areas it was almost completely absent; in others it consisted of several layers of granular cells ranging in number to seven. One region of the section showed a marked acanthosis with marked keratin production and the formation of horny plugs. In this region, the basal layer was hypertrophied, contained little pigment, and was surrounded by masses of inflammatory cells. The cells in

the acanthotic areas, and more particularly in the lower layers of the rete, showed intracellular and intercellular edema with vacuolization and widening of the intercellular spaces. In this region, the lower portions of the rete appeared infiltrated with inflammatory cells, indistinct, and blended into the inflammatory exudate. Over the remainder of the section where the inflammatory exudate was not marked, the rete appeared narrow. Its pegs were shortened and their margins indistinct from the surrounding tissue.

The papillary layer of the corium in the granulomatous areas was infiltrated with masses of cells which rarely invaded the epidermis for a short distance. The cellular collections consisted principally of histiocytes and lymphocytes, the majority seemingly being histiocytes. Polymorphonuclear cells were present, chiefly as eosinophils. Polymorphonuclear neutrophils were very rare. Collections of pigment were found in the lower papillary layer, particularly in areas where the cellular accumulation was not marked. However, collections of pigment were also found in the tips of the papillae where the infiltration was considerable. The connective tissue of the papillary and subpapillary layers appeared thickened in the noncellular areas and replaced by cells in the inflammatory ones. The lower layers of the cutis as well as the subcutaneous tissue showed few abnormalities. The connective tissue was not increased in amount. Small collections of chronic inflammatory cells were occasionally seen around hair follicles or in the vicinity of sweat glands. No marked vascular changes were seen.

#### Comment

The acute stage is marked principally by an acute inflammatory exudate, limited to the papillary and subpapillary connective tissue, which extends along the hair shafts, and consists of large numbers of lymphocytes and histiocytes. There is marked acanthosis and hyperkeratinization with plugging of the openings of the hair follicles. The stratum basalis is edematous, liquefied, and infiltrated with large numbers of inflammatory cells, mostly lymphocytes and histiocytes. Large collections of pigment are frequently found at the tips of the papillae. Such collections may also be found in the subpapillary layers. The subcutaneous tissue is not involved.

#### Case 2 (Subacute Stage)

This soldier was in the military police and was assigned to the Fifth Air Corps. He left the United States on May 25, 1943, and arrived in Brisbane, Australia, on June 13, 1943. He left Brisbane for New Guinea on August 31, 1943, and arrived in Port Moresby on September 10, 1943. He remained there until October 27, 1943, and at that time flew to Gusap, New Guinea, arriving there the same day.

*Diet.* While in Australia, he received a fully adequate diet, consisting of fresh meat, vegetables, and fruits almost every day. After arriving in New Guinea, he had a very satisfactory diet for the first 3 months. He had fresh meat almost every day and sometimes twice a day. He had apples and oranges almost every day. Subsequently, he had mainly C rations, although about once or twice a week he had fresh meat, and during this period he received practically no fresh fruits or vegetables.

*Atabrine.* Atabrine was started on August 31, 1943. Dose was 0.1 gm. daily, for the most part. For short periods of time he would take 0.2 gm. daily. Atabrine was discontinued on May 16, 1944, except when it was given therapeutically for malaria. He had had five attacks of malaria, the last being in June, 1944, and with each attack he had received atabrine. Neither malaria nor atabrine medication seemed to have any influence on the clinical course of the lichen planus. He had no symptoms to suggest sensitivity to atabrine.

*Tropical Diseases.* The patient had had malaria as described but no dengue, scrub typhus, nor tropical ulcer.

*Geographical Location.* At Gusap, New Guinea, his camp was located in the woods for the first month. He then moved out into a flat area that was covered with kunai grass. He went into swampy areas on various occasions and helped clear the jungle when the original camp was set up. He handled a moderate amount of vegetation during this period. Natives worked around their camp area and he went into native villages on a few occasions. He had worked side by side with natives but at no time did he touch them.

His present illness began after an attack of malaria in December, 1943. One week later his feet and legs began to itch. During this period he noticed that his skin seemed to bruise easily and that local infections seem to be present for long periods of time. He also noticed the development of three white sores on his lips, which had indefinite borders. On March 12, 1944, he developed swelling of his eyelids. He then developed scaling lesions over his lower legs which spread to involve most of the body. Soon he developed patchy areas which were elevated, scaled readily, and were well defined. These were grayish white. He was hospitalized on March 13, 1944, and was treated with baths and various local applications without benefit. The eruption continued to get worse. It was decided to evacuate him to the United States and he left New Guinea on May 16, 1944. Since leaving New Guinea, he estimated that he had improved 40 per cent.

*Physical Examination.* Examination revealed a generalized, diffuse, purple-red pigmentation over the skin of most of the body. The skin over his arms and legs was somewhat granular. There were three clean, shallow ulcers on the shaft of the penis. There was a patchy alopecia. There was no evidence of involvement of the mucosal surfaces.

#### Microscopic Description

The keratin layer was widened and fragmented (Fig. 2). The increase of keratin was particularly noticeable in the openings of hair follicles. The granular layer was slightly hypertrophied, particularly at the openings of the follicles. The epidermis generally was atrophic. This was particularly noticeable in the rete columns, which were frequently found to be sharpened. The basal layer was at times found to be disorganized and partly liquefied, while at other times it was somewhat hypertrophic. The papillae of the corium contained increased cells, mostly histiocytes, and scattered amounts of pigment, little of which was seen in the basal layer. The cellular infiltrate ex-

tended into the lower portions of the rete, some of the pegs of which were infiltrated and destroyed. Numerous vacuolated spaces were seen in the papillae as well as in the subpapillary connective tissue. Collections of lymphocytes were occasionally seen in the latter, and at times these showed a tendency to perivascular grouping. Slightly increased lymphocytes were seen around a few hair follicles in the lower corium.

#### Comment

The inflammatory reaction is still apparent in the subacute stage, but to a much lesser degree. Inflammatory cells in moderate numbers are still present. The outstanding lesion is the degenerative change in the stratum basalis with resultant atrophy and sharpening of the rete pegs. Considerable edema is present both in the lower epidermis and in the corium. Collections of pigment are frequently seen in the papillary or subpapillary connective tissue.

#### *Case 3 (Chronic or Healing Stage)*

This soldier was a private in the infantry and sailed for Hawaii in September, 1943. He went to the Southwest Pacific on February 2, 1944. He received atabrine during the time he was there. Two months after his arrival he developed a pruritic, vesicular eruption on the dorsal surface of the mid-finger of the right hand. Three days later an eruption of the same type began on the other hand and the lower legs. This gradually became generalized and more exudative. He entered the hospital on May 9, 1944, and was treated with starch baths and various local treatments. His disease was characterized by periods of improvement followed by periods of exacerbation. He was evacuated to the United States and arrived at Letterman General Hospital on July 29, 1944, and was then transferred to Schick General Hospital with a diagnosis of: lichen planus, hypertrophic, chronic, generalized, severe, cause undetermined.

**Physical Examination.** On examination the skin over the neck, extensor surface of the upper arms, entire forearms, and legs presented a diffuse, very heavily infiltrated, indefinitely outlined, dark brown eruption with much patchy adherent scarring. There were numerous patches of varying size and shape of the same type scattered over the body and in the groins. The mucous membranes were normal.

#### Microscopic Description

Marked hyperkeratosis without parakeratosis was present (Fig. 3). Plugging of the hair follicles by keratin was frequently seen. The epidermis varied in thickness. In some areas it appeared atrophic, while in others it was somewhat acanthotic. Variations in size of the rete pegs were seen, some appearing sharpened, while others appeared somewhat acanthotic. The basal layer at times was found to contain an increased amount of pigment. The papillary and subpapillary connective tissue appeared thickened and more homogeneous than the remaining tissue of the corium. Focal collections of inflammatory cells were seen in the subpapillary connective tissue and especially about hair follicles. A large amount of pigment was present in some of the

papillæ. Collections of inflammatory cells were seen occasionally around sweat glands and around the hair follicles in the upper regions of the corium.

#### Comment

The inflammatory reaction has largely subsided in the chronic stage. Sufficient residua are present, however, to indicate the origin of the lesion. Scattered inflammatory cells as well as occasional small foci are still present in the upper layers of the corium. Some restitution has occurred in the basal layer and some of the rete pegs are of usual width and appearance. Others, however, show persistent degenerative changes in the basal layer with consequent sharpening of their outlines. Small masses of pigment are occasionally seen in the papillary and subpapillary connective tissue. When seen in this stage, it is extremely difficult to differentiate this condition from other toxic dermatoses in tissue sections.

#### SUMMARY OF THE THREE CASES

From the above descriptions it can be seen that there are certain microscopic features which are common to the three stages. These are the hyperkeratinization and plugging of the openings of the hair follicles, the degenerative and liquefactive changes in the basal layer, and the presence of cells (their number depending on the severity of the disease) in the papillary and subpapillary connective tissue, around the hair shafts, hair follicles, and around some of the dermal glands. The changes in the basal layer are apt to be patchy, varying in severity in different regions. In the acute stage they are best discernible but even at this stage may not be distinguishable from those of other dermatoses. The cellular infiltrate consists almost entirely of lymphocytes and histiocytes, polymorphonuclear and plasma cells being uncommon. Eosinophils are occasionally seen. Accumulations of pigment are very frequently seen, as will be described below. There are no marked vascular or collagenous changes other than those that accompany acute inflammation. Edema is frequently seen in the acute and subacute stages, and is found both extracellularly and intracellularly in the prickle cell and basal layers as well as in the upper layers of the corium. Atrophy of sebaceous glands and infiltration around sweat glands are frequent.

Of all the changes noted, the one that is common to all stages is the lesion in the basal layer. This is an early as well as a late finding, and this layer of the epidermis is apparently the last to return to normality when the disease has subsided.

*Pigment.* An outstanding feature is the aggregation of pigment in the papillary and subpapillary connective tissue. These collections are

most often seen in the acute cases. Of six acute cases, 5 showed this phenomenon to a marked degree. The pigment is sometimes seen as aggregates of dark masses, most characteristically at the tips of the papillae, but frequently also in the subpapillary connective tissue. The pigment is found mostly in macrophages, although it is also seen scattered in the connective tissue. This pigment gives a negative reaction with stains for hemosiderin or hemofuscin, and gives a positive reaction with the Becker stain for melanin.\* This pigmentation is not limited to the acute cases and even in some of the chronic cases it occurs in appreciable amounts in the papillary or subpapillary connective tissue. The normal pigment of the basal layer is generally found to be increased.

#### VISCERAL MANIFESTATIONS

Early, my attention was called to the fact that, in addition to the skin lesions, some patients had general somatic disturbances. These were particularly hematologic, pulmonary, hepatic, and possibly cerebral. They varied in different patients. In the majority no such symptoms were apparent while in others they were the outstanding features of the disease. There are insufficient pathologic data concerning the pulmonary, hepatic, or cerebral findings. A description of the clinical findings will appear in a report by Lt. Col. D. J. Wilson, Chief of the Dermatology Section. Some observations have, however, been made upon the hemal and hematopoietic alterations.

#### HEMATOLOGIC FINDINGS

##### ANALYSIS OF FINDINGS

There were available 18 cases which had various blood examinations. These are summarized in Tables I to III. Changes in the cellular elements as well as in the chemical constituents of the blood were encountered. A moderate anemia was found in 12 of 18 cases examined (Table I).

\* Becker's Stain for Melanin

(*Arch. Dermat. & Syph.*, 1927, 16, 259-290)

1. Run down through water in the usual way.
2. Wash in doubly distilled water.
3. Stain in 2% silver nitrate for 2 hours (2 gm.  $\text{AgNO}_3$ , 100 cc. doubly distilled water) in dark oven,  $37^\circ \text{ C}$ .
4. Wash quickly in doubly distilled water.
5. Treat with aqueous solution of sodium thiosulfite, 1 min. (6 gm. to 300 cc.  $\text{H}_2\text{O}$ ).
6. Counterstain in Harris' hematoxylin, 2 min.
7. Dip in acid alcohol.
8. Wash.
9. Saturated solution of lithium carbonate (until tissue is a bright blue).
10. Wash in tap water and 95% alcohol.
11. Dehydrate in acetone.
12. Carbol xylol, xylol, mount in balsam.

TABLE I  
Blood Counts in Cases Studied

No.	Date	Hemo- globin	Red blood cells	White blood cells	Differential count						
					Poly.	Lymph.	Mono.	Eosin.	Late meta.	Baso.	Others
1.	8/9/44	93%	4,510,000	5,700	69	16	3	12			
2.	10/12/44	86%	4,220,000	4,950	57	43					
3.	9/30/44 10/17/44 10/24/44	103% 69% 72%	5,010,000 3,130,000 3,530,000	29,450 14,450 8,150	67 47 55	11 21 26	5	17 32 8		5	1 2 Myel.
4.	NA										
5.	10/14/44	86%	4,450,000	6,600	49	46			5		
6.	10/12/44 12/7/44	93%	4,680,000	8,250 6,850	76 65	14 28	7	3 5			1
7.	10/1/44	97%	4,840,000	8,400	60	33	1	5			1
8.	NA										
9.	10/11/44 9/6/44	90%	4,110,000	4,650 6,500	37 59	45 31	5	13 9			1
10.	11/3/44	86%	4,420,000	7,300	57	31	3	7			2
11.	10/30/44			8,900							
12.	12/7/44 1/6/45	100% 90%	4,890,000 4,030,000	7,900 7,900	46 68	50 25			4 6		1
13.	12/18/44 11/22/44	72%	3,430,000	4,700 3,500	57 45	34 43	1	8 12			
14.	12/6/44	100%	4,960,000	5,200	43	51			6		
15.	12/20/44 12/5/44	100%	4,670,000	5,400 7,400	74 79	20 16			6 5		
16.	11/22/44	97%	4,880,000	6,100	49	46	2	2			1
17.	9/4/44	70%			7,700	64	18	7	11		
18.	9/28/44	83%			5,900	65	30		5		1
19.	10/30/44 11/11/45	20% 76%	980,000 3,250,000	3,400 4,100	36 33	63 66	1	1			
20.	2/5/45	93%	4,410,000	5,100	66	28	2	3			1
21.	11/14/44 11/20/44	90% 58%	4,540,000 3,050,000	2,700 1,540	3	94 91			5 5		1 Myel.

NA=Records not available.

Of interest is the number of cases having lymphocytosis and eosinophilia in one or more counts. Twelve of the 18 cases, or 66 per cent, showed lymphocytes of 30 per cent or over, while 13 of the 18 cases, or 72 per cent, showed an eosinophilia of 5 per cent or over. The term

TABLE II  
*Determinations of Certain Chemical Constituents of the Blood*

No.	Date	Nonprotein nitrogen (mg./100 cc.)	Total protein (per cent)	Albumin (per cent)	Globulin (per cent)	Albumin-globulin ratio
1.	10/14/44		6.4	5.2	1.2	4.3
2.	9/27/44	40.4				
3.	10/21/44 10/17/44	31.36	5.34	3.18	2.16	1.47
4.	NA					
5.	10/14/44		6.2	3.95	2.25	1.75
6.	10/12/44		8.7	5.2	3.5	1.5
7.	10/1/44		8.6	5.2	3.4	1.53
8.	NA					
9.	10/11/44 10/17/44	42.46	7.8	5.2	2.6	2.0
10.	NA					
11.	NA					
12.	NA					
13.	11/22/44	27.0	5.15	2.76	2.39	1.15
14.	NA					
15.	NA					
16.	NA					
17.	NA					
18.	NA					
19.	12/5/44		6.92	4.46	2.46	1.81
20.	NA					
21.	12/10/44	43.0				

NA=Records not available.

lymphocytosis is used to designate a count of 30 per cent or over. By the term eosinophilia is meant an eosinophile count of 5 per cent or more.

Changes in the chemical constituents of the blood were encountered occasionally. These consisted particularly in a decrease in the total protein and in a lowering of the albumin-globulin ratio (Table II).

It must be mentioned that these figures cannot be considered as representative since only the more severe cases and particularly those

with some complication had complete blood studies. In a complete statistical study of unselected cases the figures would undoubtedly be much lower.

#### APLASTIC ANEMIA

Two cases had severe hematopoietic disturbance; one of these has improved markedly, while the other terminated fatally.

##### *Case 4*

The patient was a white male, 23 years old, who was admitted to Schick General Hospital on October 2, 1944, with a generalized skin eruption. Previous medical history was irrelevant. He had entered the Army on October 15, 1942, and was transferred to New Guinea in January, 1944. Two months after his arrival he developed a dry, scaly eruption on the dorsum of the right hand which eventually spread and became generalized. This condition had periods of remission and exacerbation. He was finally evacuated to the zone of the interior and arrived at Schick General Hospital on October 2, 1944, with a transfer diagnosis of dermatitis, chronic, eczematoid, generalized, severe.

Examination on admission at Schick General Hospital showed some evidence of weight loss and marked atabrine discoloration of the body. There was a patchy, fairly well outlined, erythematous, moderately infiltrative, scaly dermatitis with many small punched-out ulcerations, some bleeding, on the lower third of both legs and dorsal aspects of feet. Hemoglobin on admission was 93 per cent, and white blood cell count, 5,250. The diagnosis of lichen planus, New Guinea, was made.

On October 12, 1944, he developed a chill and on October 13, 1944, malarial parasites (*vivax*) were found in the blood. He was treated with atabrine, and completed his malarial therapy on October 20, 1944, with disappearance of parasites from the blood and remission of his symptoms. On October 30, 1944, he complained of weakness and tiredness. He appeared anemic. An examination of the blood was reported as follows: hemoglobin, 20 per cent; red blood cells, 1,280,000; white blood cells, 2,400; color index, 0.82; platelets, 70,000; differential: neutrophils, 36 per cent; lymphocytes, 63 per cent; eosinophils, 1 per cent. He was treated with transfusions, liver, and iron with considerable improvement.

Bone marrow cell count, done on January 17, 1945, was as follows: metamyelocytes, early, 1.5 per cent; metamyelocytes, late, 3.5 per cent; polymorphonuclear neutrophils, 18 per cent; polymorphonuclear eosinophils, 0.5 per cent; small lymphocytes, 65 per cent; large lymphocytes, 11.5 per cent.

The patient improved rapidly and on February 1, 1945, blood count was as follows: red blood cells, 4,650,000; white blood cells, 5,550; hemoglobin, 97 per cent; differential: neutrophils, 24 per cent; lymphocytes, 73 per cent; monocytes, 3 per cent; slight anisocytosis and poikilocytosis; reticulocytes, 1.7; platelets, 130,000. Other laboratory studies were as follows: Kahn test, negative; hematocrit, 46 per cent; volume index, 1.07; icteric index, 4.9; cephalin flocculation test, negative; hippuric acid excretion test, 3.51 gm. (normal, 2.55 to 3.3); serum protein, 6.92; serum albumin, 4.46; serum globulin, 2.46 per cent.

In summary, this was a case of lichen planus which developed symptoms and blood findings of aplastic anemia shortly after an attack of malaria for which he received an intensive course of atabrine therapy. While his blood picture had not become entirely normal, it had improved sufficiently to warrant the belief that it would become so.

*Case 5*

The patient was a white male, 33 years old, a captain, who was admitted to Schick General Hospital on November 11, 1944. Family and previous history were irrelevant. He had entered the Army on January 19, 1943.

*Present Illness.* On about April 1, 1944, approximately 7½ months before admission to Schick General Hospital, while in New Guinea, he had developed a generalized skin eruption which was diagnosed as lichen planus. He was treated with various external applications, with improvement. He continued to take atabrine up to October 1, 1944. In October, 1944, he noted gradual onset of fatigue, weakness, headache, numbness and tingling sensations of the extremities. He was admitted to the Station Hospital in the Southwest Pacific where on October 21, 1944, blood count showed: red blood cells, 1,000,000; white blood cells, 2,700; polymorphonuclear cells, 22 per cent; lymphocytes, 78 per cent; hemoglobin, 60 per cent; platelets, markedly diminished. A diagnosis of aplastic anemia was made. He was evacuated to the zone of the interior and was admitted to Schick General Hospital on November 11, 1944. Here the lichen planus was found to be largely healed. The diagnosis of normochromic anemia of the aplastic type was corroborated.

He showed no response to vigorous therapy, including pentnucleotide and repeated transfusions, developed multiple hemorrhagic phenomena, and died as a result of cerebral hemorrhage.

**Post-Mortem Findings**

The body was that of an adult white male of good development and nutrition. There were seen ecchymoses of both palpebrae and especially of the left, with marked swelling, a thin bloody discharge from the nose, small ulcers of gums, and small ecchymoses of the buccal mucosa. There were no palpable lymph nodes. Sclerae showed a slight icteric tint. There were numerous ecchymotic hemorrhages of the face, trunk, shoulders, and extremities which ranged to 2.0 cm. in diameter. No palpable abdominal masses or viscera were found. The anterior muscles of the trunk were dark red, well developed, and contained numerous large, ecchymotic hemorrhages which ranged to 3.0 cm. in diameter.

*Heart.* The visceral pericardium showed scattered small ecchymoses. The epicardium contained numerous black ecchymoses. The valves were natural. The auricular and ventricular cavities were of usual appearance. The myocardium of the left ventricle was pale brown and on section showed occasional, isolated petechial hemorrhages. The coronary vessels were patent throughout and showed slight superficial atheromatous patches. The aorta showed scattered, slight, superficial lipid deposits.

*Spleen.* The spleen was enlarged, weighing 225 gm. The surface was smooth; the capsule not thickened. The organ was soft to palpation. On section, it was seen to be congested.

*Liver.* The liver weighed 2500 gm. The capsule was not thickened, and the surface was smooth. On section, the organ had a pale gray-brown appearance. No areas of necrosis were seen. The liver was not congested. A few subcapsular ecchymoses were present.

*Gallbladder.* The gallbladder measured 5.5 by 2.2 cm. It contained yellow bile and no stones. The extrahepatic ducts were patent.

*Stomach.* The stomach was markedly dilated and contained 250 cc. of undigested food. The mucosa was studded with numerous hemorrhages varying in size from 0.1 to 0.5 cm. in diameter. There were seen also numerous brownish marks on the serosal surface, apparently from previous hemorrhages.

*Kidneys.* Weight of the left kidney was 225 gm. It was surrounded by an abundant perirenal fat, in which there was a hemorrhagic area 3.5 by 3.5 cm. The capsule stripped with ease, leaving a smooth surface. The architecture of the kidney was well maintained. The cortex over the pyramids was 0.8 cm. in thickness. The width of the pyramids was 2.0 cm. In the pelvis and upper calyces there was found a recent hemorrhage which extended to the upper portion of the ureter. The right kidney weighed 250 gm. and resembled the left in all respects. The pelvis and calyces were filled with a large hemorrhage which also extended into the upper portion of the ureter.

*Adrenals.* The right adrenal weighed 12 gm. The cut surface showed marked autolysis of the medulla. The cortex was narrow and yellow. Surrounding both adrenals in the retroperitoneal fat were large ecchymoses. The left adrenal weighed 7 gm. It was similar to the right.

*Sternal Bone Marrow.* The usual amount of marrow was obtainable. It was pale brown, somewhat more fluid than usual and contained a considerable amount of fat.

*Lungs, Pancreas, Thyroid, Pituitary Body, Small and Large Intestine, Bladder, Prostate* showed no significant gross alterations.

*Brain.* Weight of the brain was 1721 gm. The vessels on the surface of the brain were markedly congested. An area of ecchymosis, 2.0 by 2.0 cm., was seen lying within the angular gyrus on the left side. At the base of the brain a large subarachnoid hemorrhage was found covering the pons, midbrain, medulla, and the posterior portion of the cerebellar hemispheres. On section, all ventricles were found to be filled with blood. The basal hemorrhage was seen to extend into the interpeduncular space. The lateral ventricles were distended with massive hemorrhage and the septum pellucidum was ruptured. The vessels of the circle of Willis were all embedded in the hemorrhage on the surface of the brain. No definite abnormalities were seen in them.

### Microscopic Description

**Skin.** Sections of skin were cut along the line of the "Y" incision of the chest and abdomen. Small areas of hyperkeratosis were present, extending into the openings of the hair follicles. The granular layer was not well visualized. The stratum spinosum consisted of a few layers of cells. The basal layer varied in appearance. In some regions it was well outlined, in others the demarcation between the basal layer and corium was indistinct. The tips of the rete pegs were found in some regions to be partially liquefied. Some of the individual cells showed intracellular edema. A number of scattered histiocytes were present in some of the papillae and in areas of the subpapillary connective tissue. Scattered collections of pigment were occasionally seen in the papillary and subpapillary tissue, mostly in the latter. Such small masses were mostly in chromatophores, although a few granules were seen in the connective tissue itself. Small collections of lymphocytes and histiocytes were seen also in the vicinity of a few sebaceous glands and occasionally near a few sweat glands.

**Myocardium.** The myocardial muscle fibers were generally intact. A slight increase of connective tissue was occasionally seen about some of the smaller blood vessels. In one area there was evidence of a chronic focal myocarditis around an area of necrosis. This was composed of necrotic muscle fibers in which were found masses of bluish granular material. The smaller blood vessels showed no marked changes. Another section of myocardium including mitral valve showed no marked abnormalities.

**Spleen.** The splenic capsule was not thickened. The fibrous trabeculae were of usual width. The follicles were decreased in number and in size. The pulp was of usual appearance, containing numerous macrophages, a few of which were multinucleated. There was a marked increase of brown pigment (hematoxylin and eosin stain) found mostly in the macrophages. The fibrous structure of the organ was not increased in amount. The blood vessels were of usual structure. Iron stain of the spleen showed it to contain a large amount of iron in the pulp.

**Liver.** The liver capsule was not markedly thickened. Underneath the capsule, however, there were seen small areas of degeneration, fibrous tissue replacement, and chronic inflammatory cells. The last consisted principally of lymphocytes and fibroblasts. The general architecture of the liver was preserved, but changes were found in the parenchyma. These consisted of: (Fig. 4) (1) areas of separation of liver cords due to edema; (2) periportal degeneration of liver tissue and concomitant infiltration by numerous lymphocytes and some fibro-

blasts; (3) slight proliferation of bile ducts in the infiltrated areas; (4) localized collections of masses of pigment, gathered mostly in macrophages, in periportal regions. This pigment had a yellowish brown appearance (hematoxylin and eosin stain). It was found mostly in macrophages which were aggregated in foci. The pigment was granular but the individual particles varied in size. While it resembled bile pigment in some areas, it nevertheless was not seen in the bile canaliculi. Its presence in macrophages was prominently noticeable. Iron stain showed it to be negative for iron. With Becker's stain it gave the same reaction as melanin. (However, Becker's stain is not specific for melanin and apparently will also stain bile pigment.) This pigment was also found in the Kupffer cells which were very prominent throughout the liver substance. The latter also contained a considerable amount of iron-positive material.

*Kidney.* The renal capsule was not thickened. Many of the glomerular spaces were dilated, some containing fluid. The tubules were somewhat dilated, particularly the proximal convoluted ones. Some contained a small amount of frothy material. The interstitial tissue was not increased. The blood vessels showed no abnormalities. There was a large recent hemorrhage in the pelvic connective tissue and fat.

*Thoracic Lymph Node.* A thoracic lymph node contained a large amount of anthracotic material. No other pigment was seen with the hematoxylin and eosin stain. The general structure of the lymph node was maintained but the follicles were not well outlined. The blood sinuses were dilated and were occasionally seen to contain large histiocytic cells, some bearing pigment. Iron stain showed the lymph node to contain a considerable amount of hemosiderin.

*Vertebral Bone Marrow.* The bony trabeculae of the vertebral marrow were of normal appearance. There was a marked decrease in the number of cells and a corresponding increase of adipose tissue so that the marrow appeared as a mass of fat in which marrow cells were found isolated or in small groups. These were of two principal varieties: (1) small cells resembling lymphocytes; (2) larger cells containing a considerable amount of homogeneous reddish cytoplasm (hematoxylin and eosin stain) which surrounded darkly stained homogeneous nuclei, which were sometimes found to be eccentric. Multi-nucleated cells were seen only very rarely. A considerable amount of pigment was present. Iron stain showed a marked increase of iron pigment throughout the section. The appearance of the marrow was that of a marked hypoplasia with some erythroblastic regeneration. The bone marrow cell count (200 cells counted) gave: megaloblasts,

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0.5 per cent; early erythroblasts, 3.0 per cent; degenerated early erythroblasts, 1.5 per cent; polymorphonuclear neutrophils, 0.5 per cent; lymphocytes, 92.5 per cent; monocytes, 2.0 per cent (28.5 per cent degenerated cells per 100 white blood cells counted).

The sternal marrow was similar in appearance to the vertebral marrow.

*Brain.* Section of various parts of the brain showed no marked parenchymatous changes. The hemorrhages noted on gross examination were limited to the subarachnoid regions and to the ventricles.

*Blood Culture (Post-Mortem).* A hemolytic *Staphylococcus aureus* and hemolytic streptococcus were isolated.

*Final Diagnoses.* (1) Lichen planus (New Guinea); (2) aplastic anemia; (3) focal myocardial hemorrhages and necrosis; (4) bilateral pleural effusion; (5) pulmonary edema; (6) congestion of spleen; (7) hepatitis, subacute; (8) bilateral pelvic hemorrhages in kidneys; (9) subarachnoid and intraventricular hemorrhages; (10) hypoplasia and fatty change of bone marrow; (11) multiple ecchymoses of skin, voluntary muscles, diaphragm, and viscera.

#### Comment

This patient's disease began with a skin lesion which was diagnosed as lichen planus (New Guinea) about 6 months prior to his terminal illness. On about October 1, 1944, he was admitted to a station hospital in the Southwest Pacific where a diagnosis of aplastic anemia was made. This patient continued to take atabrine for the 6-month period between the onset of lichen planus and the development of anemia. On admission to Schick General Hospital on November 11, 1944, the lichen planus was found to be largely healed. The anemia had improved but he still had agranulocytosis and decreased platelet count. He showed no response to vigorous therapy, developed multiple hemorrhagic phenomena, and died as a result of a large cerebral hemorrhage.

At autopsy numerous hemorrhages were found including ecchymoses of skin, kidney, and brain. No nodular lesions were seen in the skin, but skin along the line of incision showed changes interpreted as residua of the previous disease. The marrow showed hypoplasia with predominant decrease of granulocytic elements.

Of special interest in the case was the liver lesion. There was subacute hepatitis involving chiefly the peripheral portions of the lobules, characterized by degeneration of liver tissue and accumulation of chronic inflammatory cells. In addition, isolated masses of pigment were found which were iron-negative and melanin-positive (Becker stain). The Kupffer cells also contained large amounts of pigment.

It is assumed that this pigment is bile pigment. However, there is some resemblance to the melanin pigment of the skin. With ultraviolet light the liver showed a marked greenish fluorescente. The presence of this marked fluorescence in a pathologic liver of a patient who had not had atabrine for 6 weeks prior to his death again called attention to the possibility of atabrine being a factor in this disease. Since, however, this is the only case that I have seen, no definite conclusions are warranted.

TABLE III  
*Analysis of Cases Examined  
 (Type of Lesion, History of Malaria, Presence of Anemia,  
 Lymphocytosis and Eosinophilia)*

No.	Type of inflammation in section	Malaria	Atabrine taken	Anemia	Lymphocytosis	Eosinophilia
1.	Acute	NA	+	-	-	+
2.	Acute	-	+	+	+	-
3.	Acute	-	+	+	-	+
4.	Chronic	NA	+	NA	NA	NA
5.	Subacute	+	+	+	+	+
6.	Subacute	+	+	-	-	+
7.	Chronic	+	+	-	+	+
8.	Acute	+	+	NA	NA	NA
9.	Subacute	-	+	+	+	+
10.	Chronic	NA	+	+	+	+
11.	Subacute	-	+	NA	NA	NA
12.	Subacute	+	+	+	+	+
13.	Acute	+	+	+	+	+
14.	Chronic	-	+	-	+	+
15.	Chronic	-	+	-	-	+
16.	Chronic	-	+	-	+	-
17.	Acute	+	+	+	-	+
18.	Subacute	-	+	+	+	+
19.	Chronic	+	+	+	+	-
20.	*Subacute	-	+	+	-	-
21.	Chronic	-	+	+	+	-

\* Contracted the disease in the European Theater of Operations.  
 NA=Records not available.

#### DISCUSSION

This disease is one of varied manifestations. Admittedly the most easily recognizable signs are those concerning the skin. However, many other systems of the body have been seen to be involved. The altered function of the body as shown by the occasional finding of decreased serum protein and the lowering of the albumin-globulin ratio gives further evidence of more than a local involvement of the skin. Except for its relative benignity, the disease can perhaps be considered analogous to lupus which also has visceral as well as dermal manifestations. The liver and hematopoietic systems apparently are not infrequently involved. Possibly sufficient study would indicate some involvement in even the milder cases. The occurrence of hematopoietic disturbance, although infrequent, should be kept in mind.

All of the patients investigated, with one exception, came from the Southwest Pacific area. All in this series had had atabrine, including the patient who came from the European Theater of Operations. The amounts varied, but all of them had taken the drug for some time, either in suppressive or therapeutic doses, or both. As to malaria, complete records were not available. All patients came from regions where malaria is endemic. In 18 cases records were available. Of these, 9 gave a positive and 9 a negative history of malaria (Table III). It is of interest that 7 of the 21 patients had clinical relapses with parasites in their blood during their stay at Schick General Hospital (Table III). Atabrine has been mentioned as the determining factor. It still is not clear why this condition should occur proportionately much more frequently in New Guinea than other regions where similar conditions prevail. It is possible that atabrine is only one of the factors in a chain of causes which may eventually be found in diet, climate, ingested drugs, insecticides, or infection with malaria. It would seem advisable to evacuate the patient to the zone of the interior once the disease has become manifest.

#### CONCLUSIONS

1. The disease heretofore described as lichen planus (Southwest Pacific) is one of varied manifestations, the skin being only one of the sites of localization of lesions. It occurs only in those who have been taking atabrine.
2. Although occurring most frequently, by far, in the Southwest Pacific, it has also been found in the European Theater of Operations. One such case is included in this report.
3. The skin lesion can be described pathologically as occurring in three stages which merge into one another. It resembles other lichenoid dermatoses and is easily differentiated from them morphologically only in the acute stage.
4. Visceral complications, particularly hematologic and hepatic, are encountered. Severe aplastic anemia developed in 2 of the 21 patients in the series studied.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATE

### PLATE 102

FIG. 1. Acute stage. Hyperkeratosis. Marked cellular infiltrations of the papillae and corium. Edema and degeneration of the rete. Hematoxylin and eosin stain.  $\times 85$ .

FIG. 2. Subacute stage. Edema and degenerative changes are still seen in the stratum basalis. Cellular infiltrations have decreased in amount. Collections of pigment occur chiefly in chromatophores. Hematoxylin and eosin stain.  $\times 85$ .

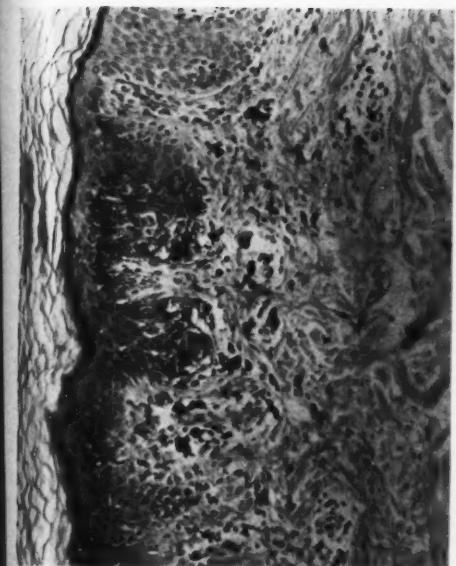
FIG. 3. Chronic stage. Hyperkeratosis and plugging of hair follicles. Involvement of basal layer is slight. Scattered inflammatory cells remain, particularly around the hair shafts. Some chromatophores are seen. Hematoxylin and eosin stain.  $\times 85$ .

FIG. 4. Liver. Collections of chronic inflammatory cells and pigment-bearing macrophages in the liver parenchyma. Becker's stain for melanin.  $\times 285$ .

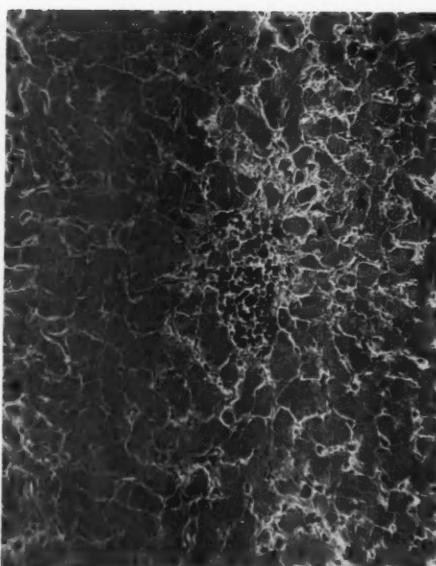
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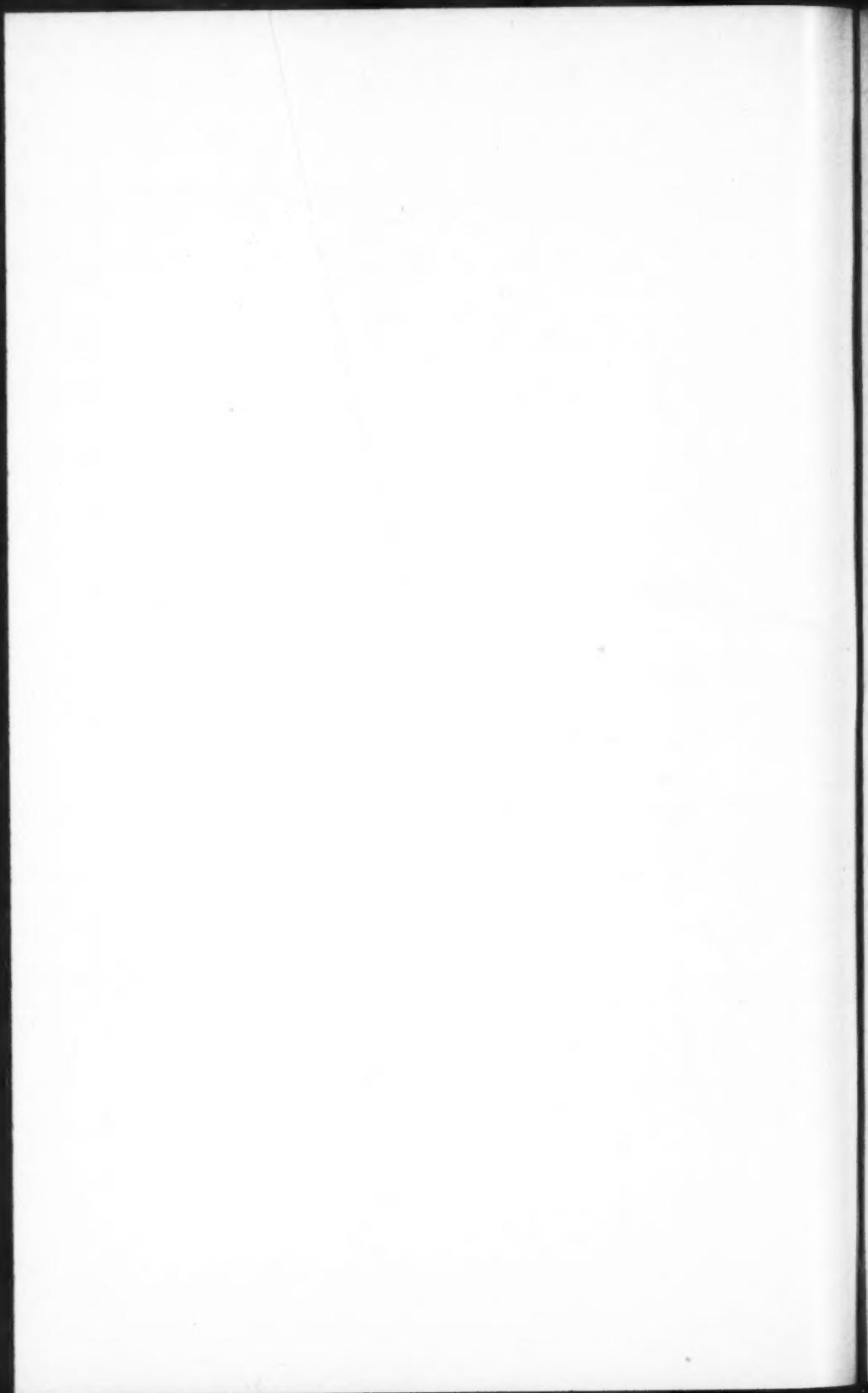


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## COEXISTENT PULMONARY ASBESTOSIS AND SARCOIDOSIS \*

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Pulmonary asbestosis, regarded as a "modern disease" by Gloyne and Merewether,<sup>1</sup> was first described by Murray<sup>2</sup> in 1900. Although Fahr<sup>3</sup> described a case in 1914, interest in this disease was not reawakened until the case of Cooke and McDonald was described in 1927.<sup>4</sup> Since that time, there have appeared in the available literature reports upon approximately 150 necropsies on cases of pulmonary asbestosis.<sup>5-12</sup> The paucity of proved cases, in comparison with those of silicosis, is not due wholly to failure to report such cases, for in large necropsy series asbestosis is apparently of infrequent occurrence.<sup>5-13</sup> Further, despite the widespread usage of asbestos products, there are comparatively few people engaged in the asbestos industry. As of October, 1944, only 19,700 people were employed in this industry in the United States.<sup>14</sup>

Much has been written about the clinical, roentgenologic, and biopsic aspects of sarcoidosis. However, because of the infrequency and relatively benign character of this disease there are only isolated detailed necropsy reports. From the available literature there have been found only 58 reports of necropsies on cases of sarcoidosis.<sup>15-21</sup> Most of these were summarized by Pinner.<sup>15</sup>

These two diseases present many clinical and roentgenographic similarities, and, also, their more frequent fatal complications are alike: pulmonary tuberculosis and cardiopulmonary insufficiency. Bronchogenic carcinoma, a frequent complication of pulmonary asbestosis, has not, however, been described as associated with sarcoidosis. Likewise, there has not been a previous description of asbestosis with coexistent sarcoidosis. It is the purpose of this report to present the findings in such a unique case, the only example of either pulmonary asbestosis or sarcoidosis in a series of 1870 necropsies done at this hospital.

### REPORT OF CASE

The patient was a white male, 42 years of age. Subsequent to hemorrhoidectomy in December, 1943, he had noticed that slight activity produced shortness of breath. He did not experience nocturnal dyspnea and he was able to lie flat in bed without respiratory difficulty. There was no history of cough, hemoptysis, or cardiac embarrassment. Notwithstanding a good appetite and the absence of gastric

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symptoms, there was a weight loss of 22 pounds from December, 1943, to March, 1944, at which time he presented himself for medical care.

The patient had worked in an asbestos plant for 25 years, the last 10 years having been in a supervisory position. During this entire time he had worked in one department in which asbestos pipe was made. There was a slight but appreciable dust hazard associated with the sawing and splitting of the dried asbestos pipe, despite precautionary exhaust ventilation. The total time the patient had spent upon this final operation of sawing was unknown; nor was it learned whether he had been negligent in using the provided respirators. To the company's knowledge, this was their first case of asbestosis.

Physical examination revealed the following findings: Temperature, 37° C.; respiration, 22 per minute; arterial blood pressure, 105/70 mm. Hg; height, 170 cm.; weight, 67 kg. The chest was of increased anteroposterior diameter. Respiratory excursions were equal but decreased. The percussion note was resonant and auscultation revealed fine râles over the bases of the lungs, posteriorly. There were no evidences of cardiac enlargement, irregularity, or decompensation. Cyanosis and clubbing of the fingers were absent. The liver was barely palpable.

Report on the roentgenogram of the chest (Fig. 1) was as follows: "The bony framework is normal. The trachea is in the midline. The hilum shadows are moderately enlarged, bilaterally. One small calcified area is present in each hilum. There are numerous small nodular densities scattered throughout both lung fields, especially throughout the lower lobes. There is some confluence of these densities in the left lower lobe. Emphysema is present."

Examination of the blood showed erythrocytes, 5.5 million; leukocytes, 6.6 thousand; 73 per cent neutrophiles; 21 per cent lymphocytes; 5 per cent monocytes; 1 per cent eosinophils; sedimentation rate, 26 mm. No abnormality was found in the urine. The vital capacity, 2200 cc., was 51 per cent of normal. Tuberculin tests were not done.

The patient was seen at regular intervals and his only complaint was increasingly severe exertional dyspnea. A roentgenogram of the chest 4 months after the initial chest film revealed no new findings. Although the patient greatly limited his activities, dyspnea became progressively more severe so that eventually, even at bed rest, there was extreme air hunger. At no time were there evidences of cardiac failure. He died approximately 11 months after the onset of symptoms, apparently from respiratory failure.

#### *Autopsy Findings*

The necropsy was performed 5 hours after death. Superficially, there was considerable decrease in the subcutaneous tissues and the body musculature. There was no clubbing of the nailbeds or dependent edema. The mediastinum was in the midline. Each lung completely filled its hemithorax and extended far into the anterior mediastinal space. The domes of the diaphragm, anteriorly, were at the level of the fourth interspace and fifth rib, right and left respectively.

The lungs were encased in markedly thickened, tough, yellowish white, generally fused pleurae. The interlobar fissures were obliterated by easily broken adhesions. Lobation was normal. Hemorrhagic fibrinous material, present over the posterolateral aspect of the left lower lobe, loosely bound the thickened parietal pleura to the lung in this area.

The frontal section of the left lung (Fig. 3) revealed coarse, lacy, tannish brown, hypercrepitant tissue throughout both lobes. Innumerable slightly elevated, grayish green, irregular, firm nodules, 1 to 2 mm. in diameter, were present throughout the lung. Thin, radiating, fibrous bands surrounded and connected these nodules. Also, slightly thickened pleural septa extended into the lung substance for variable depths. In the lung tissue about the bronchi of the second and third interspaces these nodules were somewhat confluent and a similar change was noted in the subpleural tissues for a depth of 3 to 5 mm. Generally, these nodules, present in moderate numbers, were separated by wide zones of dry emphysematous lung tissue studded by numerous minute, grayish tubercles. Dissection of the bronchi of the lower lobe disclosed that they were moderately dilated, cylindrically and saccularly, and lined by glistening white mucosa.

In the right lung the same changes were observed as were present throughout the left. However, the grayish green, irregular nodules tended to be more numerous, larger, and more confluent. This was particularly true of the anterior portions of the lower and middle lobes. Also, the nodules were connected by thicker grayish black and grayish white interlacing bands of fibrous tissue. Extending deeply into the lung substance, thickened pleural septa communicated with the fibrous tissue in and about the clusters of tubercles. Except for more pronounced dilatation and thinning of the mucosa, the bronchi of the right lung were similar to those of the left.

The tracheobronchial lymph nodes were moderately enlarged and on section consisted of dense, rubbery, anthracotic centers and thin rims of yellowish white tissue. Calcification was not grossly demonstrable.

The embalmed heart weighed 280 gm. and had the following measurements: tricuspid valve, 120 mm.; pulmonary valve, 80 mm.; mitral valve, 85 mm.; aortic valve, 65 mm.; right ventricle wall, 3 to 8 mm.; left ventricle wall, 15 mm. The greatest transverse cardiac diameter was 13.5 cm. (The estimated normal heart weight on the basis of body length is 317 gm., plus or minus 40.<sup>22</sup>) The tricuspid/aortic valve and pulmonic/aortic valve ratios were 1.84 and 1.23, respectively. (These normally should be 1.68 and 1.05, respectively.<sup>23</sup>) The right ventricle was dilated and its columnae carneae and papillary muscles were more prominent than usual. No mural thrombi were demonstrable and the valvular endocardium was normal.

The enlarged spleen was of normal configuration and measured 18 by 9 by 6 cm. It was covered by a smooth capsule and the splenic

substance was firm and purplish red with normal markings. No tubercles were seen. The liver measured 22 by 16 by 10 cm. The remaining organs showed passive hyperemia and moderate generalized arteriosclerosis.

#### *Microscopic Findings*

Throughout the lung there was a conspicuous linear, interlacing, peribronchial and septal pulmonary fibrosis (Fig. 2). This was particularly prominent in the subpleural tissues. The intervening lung tissue was moderately emphysematous. Innumerable tubercles were present in the linear and peribronchial fibrotic areas and were present to a lesser extent in the walls of the respiratory bronchioles and the adjacent alveolar walls. Generally, these tubercles were of two types: sarcoidal and foreign body granulomas. The former predominated by approximately ten to one. Tubercles of these types were intimately associated and, in addition, many intermediate types were presented.

The sarcoidal tubercles (Fig. 4) were free of caseation, contained no demonstrable organisms and were, for the most part, in the same stage of development; however, a minimal number presented some peripheral fibrosis and there was an occasional, coarse, collagenous ball. Generally, the tubercles were sharply demarcated, surrounded by delicate reticulum, and did not present peripheral rims of lymphocytes. They consisted of peripherally arranged epithelioid cells surrounding central, loosely arranged epithelioid and monocytic cells. Giant cells were, for the most part, centrally located and often comprised over half of the bulk of the nodule. The giant cells appeared to be of two types: Langhans' cells and foreign body giant cells, with the former predominating. In many of the Langhans' cells there were numerous small vacuoles, each containing a pink, round body. Other Langhans' cells contained large, clear vacuoles; and, rarely, in those cells containing one large vacuole there was present an "asteroid" body, an intensely eosinophilic stellate mass, 15 to 20  $\mu$  in diameter (Fig. 5).

More frequently, the Langhans' cells contained round, oval, or suggestively budding, intracytoplasmic bodies of Schaumann,\* 25 to 50  $\mu$  in diameter. Rarely, these bodies appeared to lie outside of giant cells, and some enclosed irregular yellowish material (Figs. 6 and 7). These bodies stained blue with hematoxylin and in ferrocyanide preparations were strongly positive for iron. Dr. Leroy U. Gardner,<sup>24</sup> who also studied this case, stated that these bodies stained "red with acid fuchsin of van Gieson-Weigert instead of black like elastic tissue"

\* Schaumann, J. On the nature of certain peculiar corpuscles present in tissue of lymphogranulomatosis benigna. *Acta med. Scandinav.*, 1941, 106, 239-253.

and that "von Kossa's calcium stain is negative." Re-study of appropriately stained sections revealed, as pointed out by Gardner, that the Schaumann bodies did stain red; however, a moderate number also contained calcium in variable degrees, as demonstrated by von Kossa's stain. An occasional giant cell contained one or more clefts suggestive of cholesterol crystals. More frequently, however, doubly refractile, irregular spicules, plaques, and conchoidal masses were observed in giant cells. These doubly refractile masses were often about, or in, the Schaumann bodies, particularly the smaller and partially calcified forms.

The foreign body tubercles were indefinitely demarcated and consisted of rather closely packed, indefinitely arranged, large monocytes, and one or more foreign body giant cells. These tubercles, for the most part, were within the dense zones of fibrosis. Some, however, were present in alveoli and respiratory bronchioles. Golden yellow discoid, verruciform, and incompletely segmented asbestos bodies, many of which were in giant cells, were observed in and about the nodules (Fig. 8). Asbestos bodies, singly or in clusters and in moderate numbers, were present also in the dense fibrotic areas (Fig. 9) and occasionally within alveoli (Fig. 10). Rare, laminated, calcified masses, enclosing apparent asbestos bodies (Fig. 11) and other bodies which appeared to be of the Schaumann variety, were present in the linear fibrotic bands. Asbestos bodies were also encountered in and about the sarcoidal tubercles and in the associated sarcoidal giant cells of both varieties, but more frequently in those of foreign body type. Iron preparations clearly demonstrated the bizarre forms of the asbestos bodies.

In many areas it was difficult to distinguish between the two types of lesions. This was particularly true throughout the subpleural region where both the lesions and asbestos bodies were more numerous, clustered, and embedded in a dense matrix of collagen, masses of coarse elastic fibers, and fine reticulum.

The larger bronchi were remarkable only for slight chronic inflammation. The bronchioles and respiratory bronchioles, embedded in dense collagen and surrounded by tubercles, were moderately dilated and presented conspicuous focal squamous metaplasia and moderate chronic submucosal inflammation. In the subpleural regions where the asbestotic fibrosis and the granulomatous reaction were most intense, the bronchioles were irregularly dilated and lined by alternating strips of tall columnar and squamous epithelium. Only a few bronchioles contained neutrocytic exudate. The respiratory bronchioles were generally constricted, surrounded by masses of elastica, and

many contained asbestos bodies and the associated granulomatous reaction. Within the peribronchial fibrous tissue there was a moderate amount of hemosiderin in linearly disposed granules, and fine lipoid droplets. The small pulmonary arteries and arterioles presented slight intimal thickening, and those in the subpleural zone were surrounded by thick collars of elastic fibers.

The intervening alveoli were moderately dilated, the capillaries were congested, and there was a slight increase in collagen in the alveolar walls bordering the fibrous masses. Focally, clusters of alveoli contained lipoid-laden macrophages. "Heart lesion cells" were infrequent.

Sections of the pleura revealed dense, laminated, and oval fenestrated bundles of collagen. Focally, there were indefinitely demarcated nodules which consisted of circularly disposed lamellae of collagen. Superficially, the pleura presented slight fibroblastic activity and an occasional perivascular accumulation of lymphocytes and monocytes, some of the latter occasionally containing hemosiderin. No asbestos bodies were observed. The pleura over the left lower lobe, in addition, bore organizing fibrinous exudate on its visceral aspect.

Sections of the tracheobronchial lymph nodes presented a repetitious pattern of sarcoidal tubercles with almost complete replacement of the lymphoid tissue. Throughout the nodes there were minimal diffuse fibrosis and several nodular masses of coarse collagen. The tubercles were similar to those in the lung as to structure and stage of development. Inclusions of Schaumann were not observed and only a rare "asteroid" was present. Asbestos bodies were not identified. A moderate number of hemosiderin-containing macrophages were present in the remaining lymphoid tissue.

Similar sarcoidal tubercles were present to a slight degree in the spleen and liver, and to a lesser extent in the kidneys, diaphragmatic muscle, and the right and left ventricular myocardium. These sarcoidal tubercles, however, were not as compactly arranged as those in the lung and tracheobronchial lymph nodes, and were surrounded by and permeated by lymphocytes. "Asteroid bodies" and Schaumann bodies were not present in the giant cells of these tubercles. No asbestos bodies were found. Those in the right ventricular myocardium were associated with considerable fibrosis.

The results of chemical and spectrographic analysis of lung tissue, performed under the direction of Dr. Leroy U. Gardner,<sup>24</sup> are presented in Table I.

The final diagnoses were: Moderate pulmonary asbestosis; extensive sarcoidosis of pulmonary and tracheobronchial lymph nodes; marked chronic pulmonary emphysema; slight sclerosis of the small

arteries and arterioles in the lungs; marked nodular obliterative pleural fibrosis; focal organizing fibrinous pleuritis; minimal sarcoidosis of the heart, liver, spleen, and kidneys; right ventricular cardiac dilatation and relative right ventricular hypertrophy; acute passive hyperemia of the viscera; slight cirrhosis of the liver; slight generalized arteriosclerosis; minimal focal chronic adrenalitis and nephritis; chronic posterior urethritis and interstitial prostatitis.

TABLE I  
*Chemical and Spectrographic Analysis of Ash  
(Dry Tissue, Approximately 14.1% of Moist Tissue. Ash, 6.70% of Dry Tissue.)*

As oxides (except Cl)	As elements			Arbitrary scale of relative amounts
	Chemical analysis	Chemical analysis	Spectrographic analysis	
Cu, Ag, Hg	Per cent	Per cent		
Pb, Bi, Cd	<0.15	Na	4	75
Mo		K	36.7	100
SiO <sub>2</sub>	2.76	Sr	None found	1.5
Fe <sub>2</sub> O <sub>3</sub>	8.03	Ba	None found	5
Al <sub>2</sub> O <sub>3</sub>	0.37	Ca	2.1	80
BeO	None found	Al	0.2	50
ZnO	0.39	Mg	0.7	75
MnO	0.03	P	8.0	60
CaO	2.92	Si	1.3	100
MgO	1.18	Fe	5.6	75
BaO	None found	Mn	0.02	3
SrO	None found	Ti	None found	5
TiO <sub>2</sub>	None found	Cu		25
V <sub>2</sub> O <sub>5</sub>	None found	Ag		2
Cr <sub>2</sub> O <sub>3</sub>	0.07	Sn		3
NiO, CoO	<0.05	Cr	0.05	3
Na <sub>2</sub> O	5.44	B		1
K <sub>2</sub> O	44.40	Be	None found	0
P <sub>2</sub> O <sub>5</sub>	18.42	Pb		25
Cl	4.61	Zn	0.3	5
CO <sub>2</sub>	Present	Bi		10
		Pt		3
Total	88.82	Cl	4.6	0

### DISCUSSION

Clinically, in view of the significant history of exposure to asbestos, the possibility of sarcoidosis was never entertained. In retrospect, the rapidly progressive, disabling dyspnea, unaccompanied by evidences of enlargement of the right heart or cardiac failure, should have aroused suspicion that there was a concomitant pulmonary lesion. Asbestosis alone is not usually accompanied by such profound, rapidly developing, respiratory embarrassment. In this case, however, there were no collateral clinical evidences of sarcoidosis. It would seem that a clinical diagnosis of coexistent asbestosis and sarcoidosis would be justified only by biopsy of a lymph node or a skin lesion to demon-

strate sarcoid lesions and the discovery of asbestos fibers in the sputum, with a history of adequate exposure to asbestos fibers and roentgenographic evidences of diffuse pulmonary fibrosis. Asbestosis of the degree observed, alone should not have caused death, and sarcoidosis has generally been regarded as a benign process. Reisner,<sup>18</sup> however, on the basis of his observations on cases of pulmonary sarcoidosis, stated "that one is not justified in assuming too confident an attitude regarding the ultimate outcome." This statement is particularly true when, as in this case, sarcoidosis complicates pre-existing pulmonary disease.

Pathologically, there were evidences of right heart strain in that there was marked dilatation of the right heart, evidenced by increased tricuspid and pulmonic/aortic valve ratios and slight passive hyperemia of the viscera. The total heart weight, however, on the basis of body length,<sup>22</sup> was normal. As determined by the ratio of the left and right ventricular weights, it has been shown that there may be considerable relative right ventricular hypertrophy without an increase in the total heart weight. However, relative right ventricular cardiac hypertrophy in Higgins' series<sup>25</sup> was not usually accompanied by evidences of right ventricular failure. In view of the significant dilatation of the right side of the heart and the slight sclerosis of the pulmonary arterioles, there was, in all probability, some degree of pulmonary hypertension in this case. However, in the absence of an increase in total heart weight and in the absence of evidences of chronic passive hyperemia of the viscera there was probably no, or insignificant, exaggeration of air hunger due to heart failure.

It has been suggested that dyspnea in the pneumoconioses is due to capillary and arterial blockage by the fibrotic process. This, in all probability, is true to a variable degree in those persons with severe fibrosis of the conglomerate type with attendant extreme chronic emphysema. This hypothesis, however, does not explain the severe dyspnea that is seen in occasional cases of diffuse miliary studding of the framework of the lung by silicotic, tuberculous, sarcoidal, or neoplastic tubercles. It may be that the mechanism of dyspnea in such instances is due to irritation of the vagus nerve endings with reflex stimulation of the respiratory center (Hering-Breuer reflex). In view of the equivocal evidences of hypertrophy of the right heart in this case, mechanical obstruction to the blood flow would not appear to be the responsible factor but, more likely, because of the diffuse active inflammatory process throughout the lungs, the Hering-Breuer reflex was exaggerated. Presumably, there was either a severe re-

spiratory alkalosis or acidosis. Tissue changes suggestive of alkalosis, such as calcification of the renal tubules, were not found.

Microscopically, there was some difficulty in differentiating the two types of tubercles since there were many sarcoidal tubercles which contained asbestos fibers, and tubercles of indeterminate type, not containing fibers or inclusion bodies, were sometimes seen. It was difficult to determine how much of the fibrosis was due to asbestosis. Morphologically, since the majority, by far, of the sarcoidal tubercles were without evidences of fibrosis and apparently of the same age, it is suggested that this process was engrafted upon an established asbestosis. Further, on the basis of Gardner and Cummings' <sup>26</sup> experimental studies on asbestosis, the marked peribronchiolar fibrosis with sequestered asbestos bodies, the marked pleural fibrosis and pleural septal fibrosis, and the metaplasia of the bronchiolar epithelium indicate that the asbestosis was well established and over 700 to 800 days old. Dr. Leroy U. Gardner, who kindly examined the material, stated: "In comparison with our other material the pigmented foci in your case seem to show more fibrosis and less localized emphysema. Histologically, this can probably be explained by the presence of sarcoid nodules within the asbestotic zones of reaction. I would infer that in your case the two conditions developed more or less simultaneously, but that probably the asbestosis was present to some degree before the sarcoid appeared. This opinion is based upon the occurrence of asbestos fibers and other iron-containing particles in the interior of the tubercle-like nodules and in some cases within the giant cells themselves. The number of asbestos bodies is smaller than seen in many cases."

Inclusions of the Schaumann variety, found only in the lung, occurred in 4 per cent of the giant cells. Some of these enclosed golden-yellow, irregular bodies suggesting asbestos bodies, but similar to organic material previously described within such bodies. Yet there were definite asbestos bodies enclosed by similar dark blue material. Schaumann inclusions have been described in only 4 per cent of the reported necropsies on sarcoidosis as summarized by Rubin and Pinner,<sup>17</sup> who did not regard these inclusions as specific for sarcoidosis. Rich,<sup>27</sup> who was impressed by the frequency of Schaumann inclusions in sarcoidal lesions and by their absence in unequivocal tuberculous lesions, noted that Metchnikoff reported the presence of calcified inclusions in the hyperplastic tuberculous lesions of experimentally infected Algerian rats. Kraus<sup>20</sup> stated that the presence of calcified inclusions was a feature not found in any known granuloma except

sarcoidosis. Gardner<sup>24</sup> pointed out that, in his sarcoid material, these bodies, regarded by many to consist of calcium or calcified remnants of elastica, do not, by the von Kossa method, contain calcium, but, by the ferrocyanide method, give a strong reaction for iron. Only a moderate number of the Schaumann bodies observed in the present case were either wholly or partially calcified, yet all gave a strong reaction for iron. Studies on sarcoid lesions of lymph nodes and spleen from another case revealed only a few iron-staining noncalcified Schaumann bodies. The presence of doubly refractile, nonlipoid substance in giant cells and frequently in close relation to Schaumann bodies has not been emphasized in the literature on sarcoidosis. It has been noted, however, that colorless and yellowish tinged refractile material is often enclosed by the Schaumann body. The fact that these masses are frequently doubly refractile has not been stressed. It has been suggested that these enclosed masses represent disintegrating elastica; however, van Gieson-Weigert stains do not confirm this suggestion. The origin of this refractile and doubly refractile material is not known. Being in and about many of the small, partially calcified bodies, this doubly refractile material appears to be associated with the development of the Schaumann body. The larger and more densely stained bodies were not as frequently associated with visible doubly refractile substance. However, fractured and fragmented, apparently old, Schaumann bodies, as seen in control sarcoid material from lymph node and spleen, usually contained moderate amounts of doubly refractile substance. Apparently then, the Schaumann body, which stains blue with hematoxylin and red with acid fuchsin, is formed in response to doubly refractile, nonlipoid substance and initially is impregnated by iron and later, in amounts demonstrable by von Kossa's stain, by calcium.

Wolbach,<sup>21</sup> in 1911, Jadassohn, in 1919,<sup>28</sup> and Friedman,<sup>19</sup> in 1944, have described a peculiar intracellular body in cases of sarcoidosis. This body, stellate in shape, varies in size up to 25  $\mu$ , generally lies in an intracytoplasmic giant cell vacuole, and stains intensely with acidophilic stains except the central area which is basophilic. Wolbach described them as lying free in tissue spaces, in endothelial leukocytes, and in giant cells. Friedman found such bodies in only 6 to 8 per cent of the giant cells in his case. Both investigators attempted to determine the chemical structure of this stellate body by specific stains; however, they were unsuccessful. Both considered the possibility of its being an extraneous organism, although questionable. Wolbach regarded it as a nonspecific biochemical alteration of the cytoplasm. He was never able to demonstrate stellate bodies in other

material and decided that they were not similar to inclusions sometimes seen in cases of sarcoma. Friedman regarded these bodies as nonspecific but highly characteristic of sarcoid lesions. Friedman proposed that these bodies be called "asteroids," but perhaps it would be better, eponymically, to call them Wolbach's asteroids. They have been described in 7 cases of sarcoidosis, and never in association with the Schaumann calcified inclusion body. In the present case asteroids were present in approximately 1 per cent of the giant cells in the lungs and tracheobronchial lymph nodes. Definite transition stages of asteroid formation were suggested by the presence of spicules on the pink, coccoid, intravacuolar, intracytoplasmic bodies, particularly in those giant cells in which the small vacuoles were clustered and disintegrating. In addition, an occasional Wolbach's asteroid, instead of lying in a large, clear vacuole, was surrounded by agminated ruptured vacuoles. In view of the presence of similar pink, coccoid, intravacuolar bodies, similar asteroids, and the same suggestive stages of asteroid formation in the giant cells of talcum powder granuloma, as observed in one case in this laboratory, these giant cell cytoplasmic changes must be regarded as Wolbach originally suggested, nonspecific biochemical cytoplasmic alterations. In addition, such an asteroid is depicted in the giant cells of lepromous lesions by Mallory<sup>29</sup> who called them "spiculated" bodies. No transition stages between Wolbach's asteroids and Schaumann's inclusions were even remotely suggested.

The pathogenetic relationships of asbestosis and sarcoidosis are dependent upon the chronologic development of the lesions and the nature of the causative agents. Historically and histologically, in this case, it is most likely that asbestosis preceded the development of sarcoidosis. The predominant localization of the sarcoidal tubercles within asbestotic zones of fibrosis with attendant morphologic modification of both lesions, as evidenced by asbestos bodies within sarcoidal tubercles and lesions of indeterminate type, would suggest an analogy to the intimate relationship existent between tuberculosis and the pneumoconioses. It must be remembered, though, that even in uncomplicated sarcoidosis the lesions occur in the framework of the lung, and therefore the morphologic relationships of the two may be coincidental. This would be in agreement with those who believe that morphologically sarcoid is not reconcilable with tuberculosis. However, to those who regard sarcoidosis as a peculiar form of tuberculosis, this case then would be one of asbestosis with superimposed non-caseating tuberculosis.

The authors are indebted to Dr. Leroy U. Gardner who critically examined the gross and microscopic material of this case and supplied the chemical analysis.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

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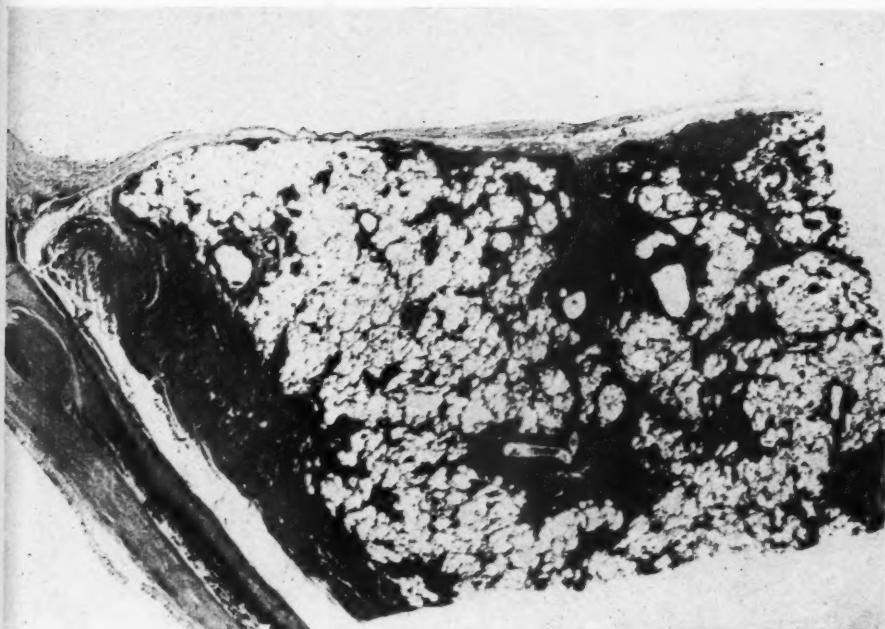
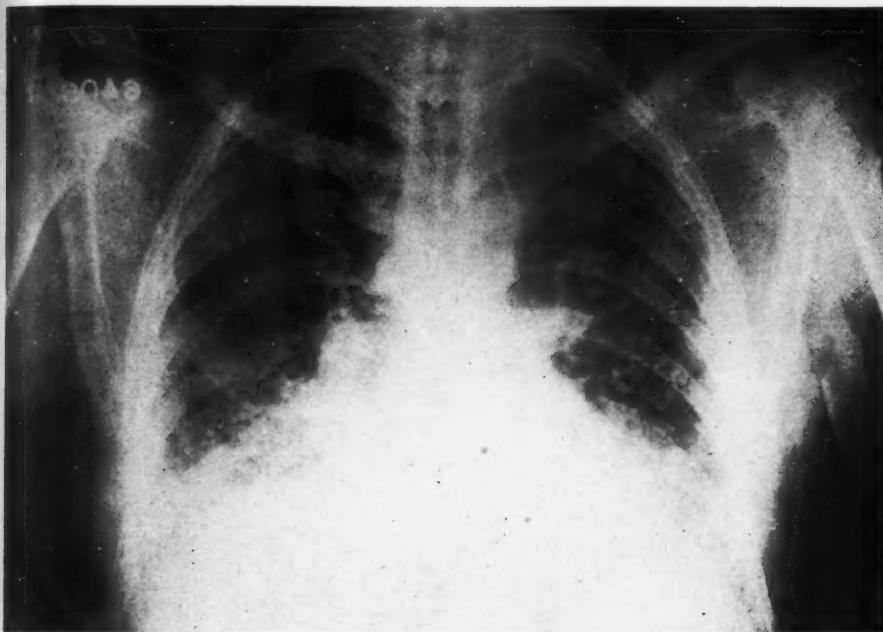
### PLATE 103

FIG. 1. Initial roentgenogram of the chest.

FIG. 2. Photomicrograph of lung and adherent pleura showing subpleural, septal, peribronchiolar, and marked and focally nodular pleural fibrosis.  $\times 4$ .







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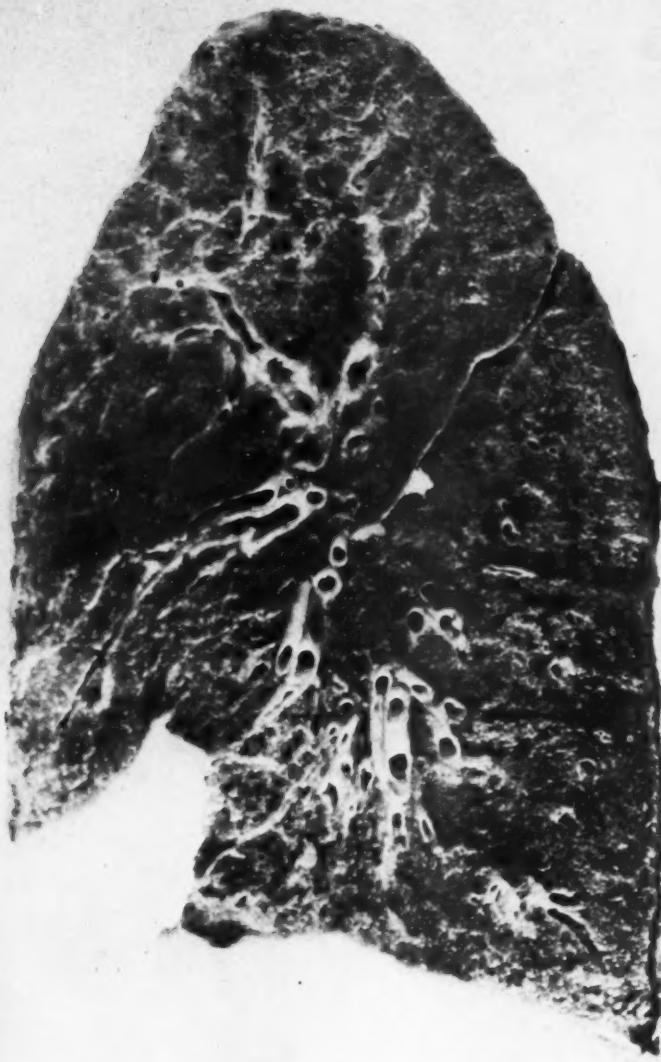
Coexistent Asbestosis and Sarcoidosis

PLATE 104

FIG. 3. Frontal section of left lung.







3

Skavlem and Ritterhoff

Coexistent Asbestosis and Sarcoidosis

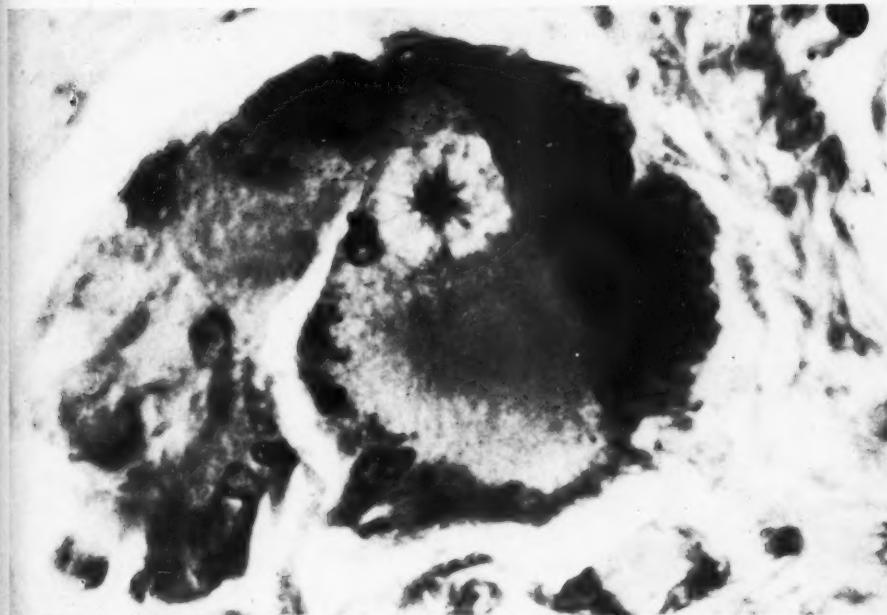
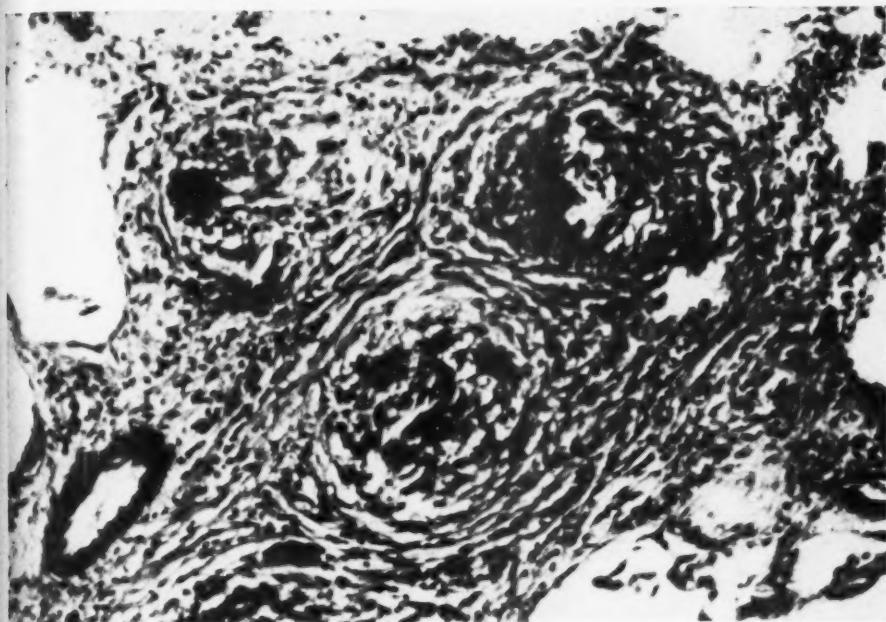
PLATE 105

FIG. 4. Lung, showing a cluster of sarcoidal tubercles.  $\times 160$ .

FIG. 5. Lung. The giant cell which nearly fills the field contains an "asteroid" in an intracytoplasmic vacuole.  $\times 1090$ .







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Coexistent Asbestosis and Sarcoidosis

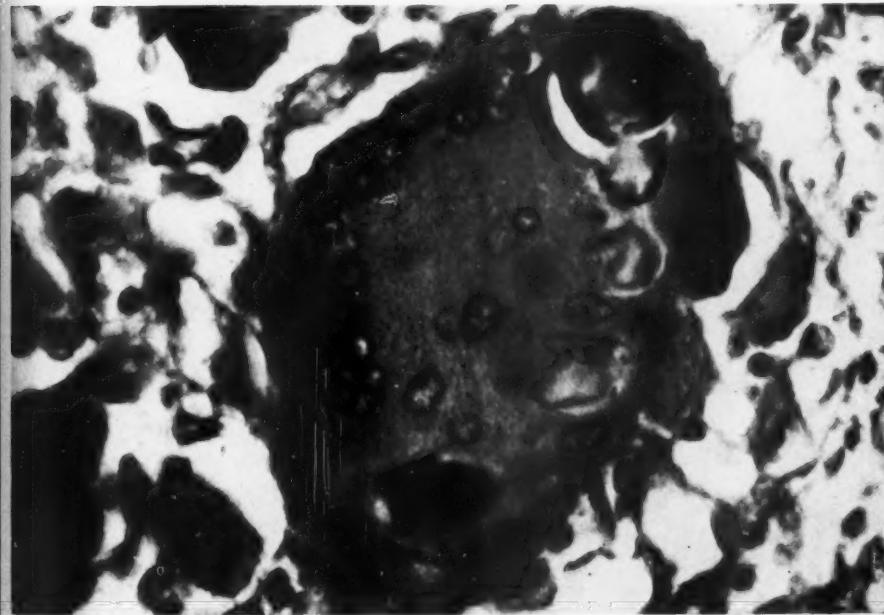
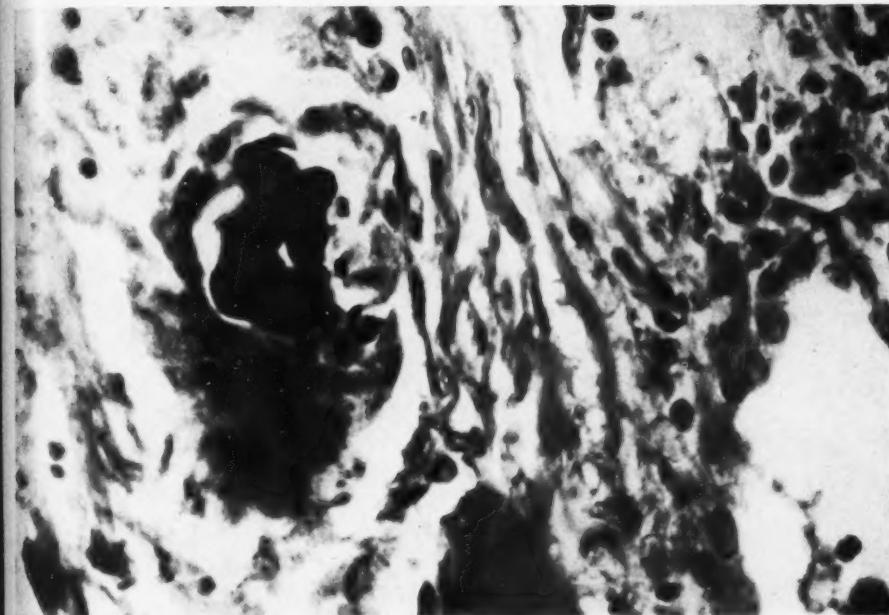
PLATE 106

FIG. 6. Lung. An inclusion of Schaumann encloses an oval yellow body. With polarized light, doubly refractile material surrounds this calcified mass.  $\times 725$ .

FIG. 7. A giant cell from the lung with inclusions of Schaumann.  $\times 725$ .







Skavlem and Ritterhoff

Coexistent Asbestosis and Sarcoidosis

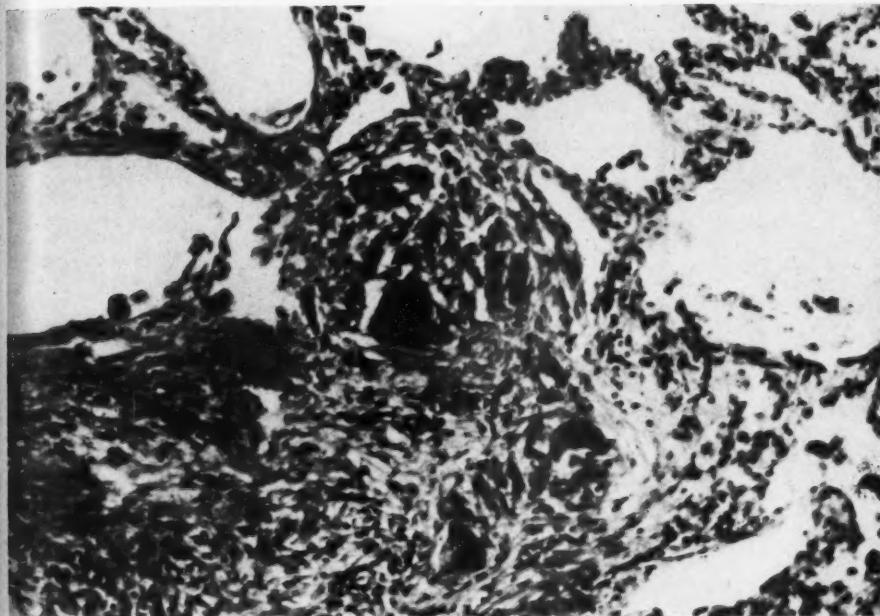
PLATE 107

FIG. 8. Lung with tubercles of foreign body type. The central tubercle has an asbestos body at its periphery.  $\times 160$ .

FIG. 9. Lung showing asbestos bodies and clusters of hemosiderin-laden macrophages within an area of fibrosis.  $\times 725$ .







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Coexistent Asbestosis and Sarcoidosis

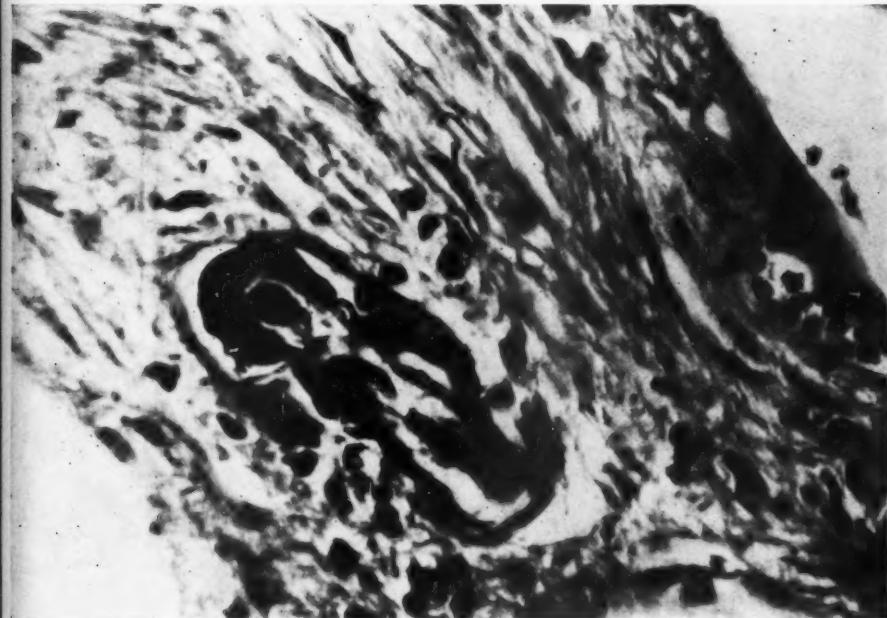
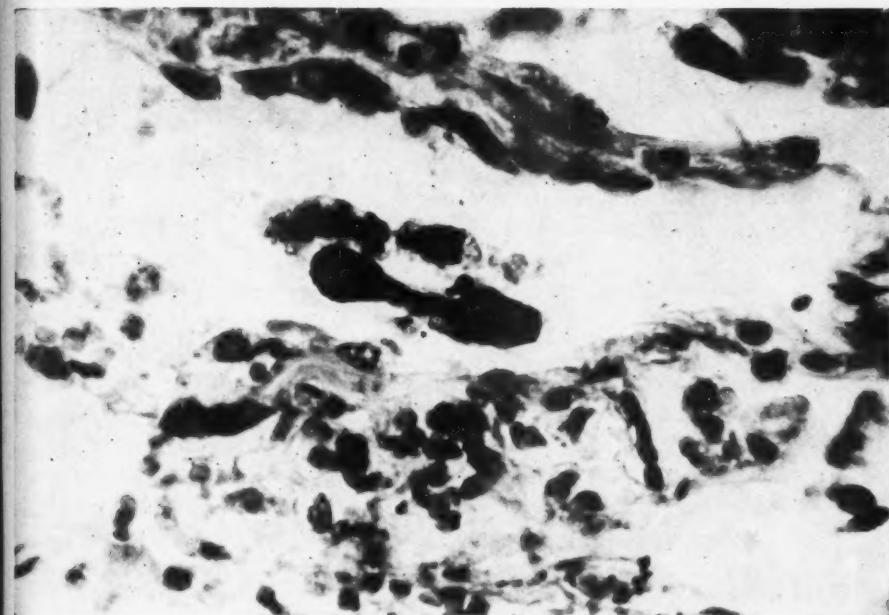
PLATE 108

FIG. 10. Lung. An asbestos body is shown in an alveolus.  $\times 725$ .

FIG. 11. Lung showing an asbestos body encrusted with iron and calcium.  $\times 725$ .

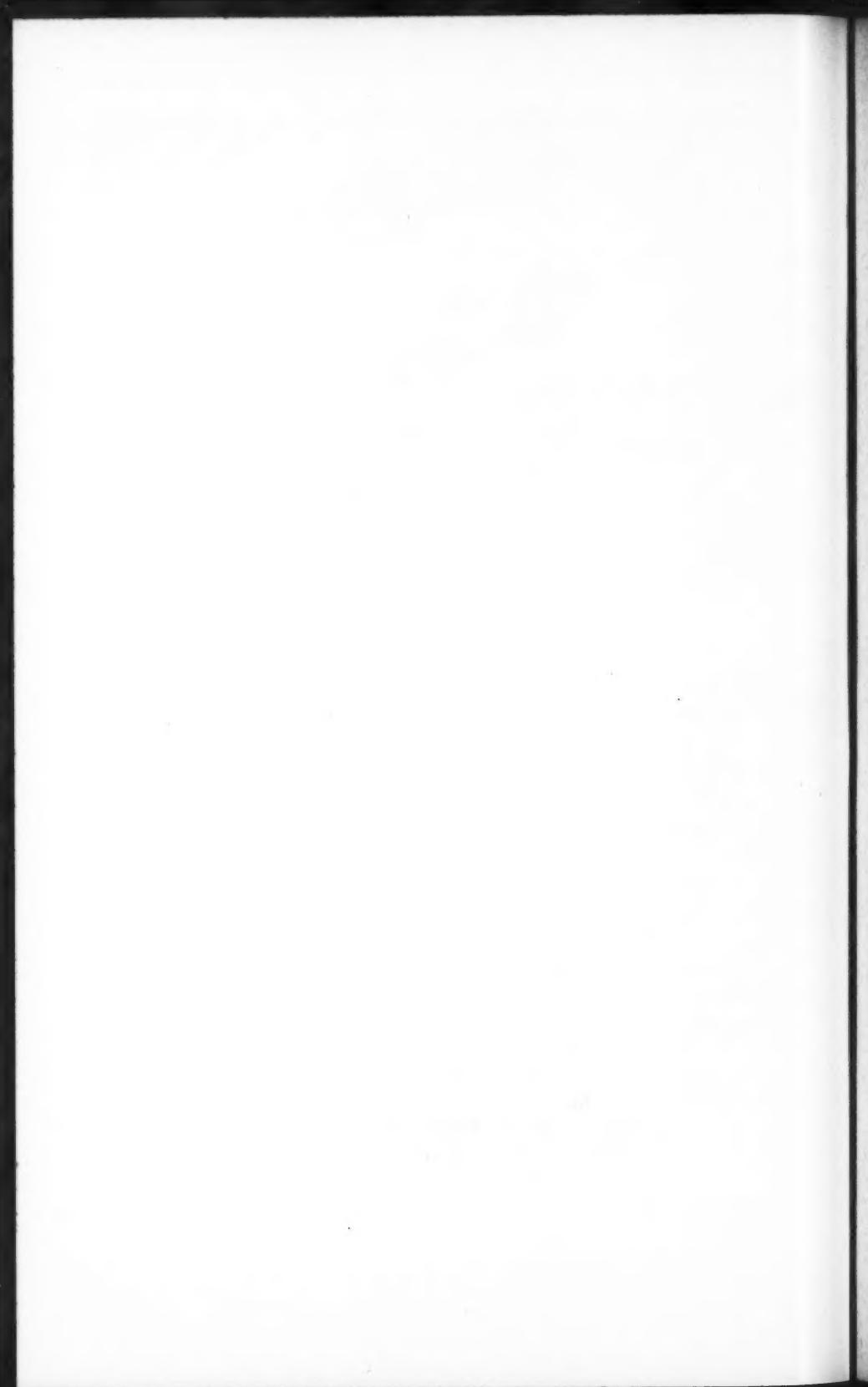






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Coexistent Asbestosis and Sarcoidosis



## XANTHOMATOSIS OF THE ARTERIAL MEDIA IN A DOG \*

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At a recent routine necropsy a dog was found to have xanthoma-like lesions affecting the media of the muscular arteries and some of the elastic arteries. In many of these vessels, fibrous connective tissue proliferation and deposition of iron-containing material were associated with lipid deposit and foam cell formation. In the few reported cases of vascular involvement in human xanthomatosis, the lesions were confined to the intima, and a primary, xanthoma-like, medial localization has never been described in any species, either spontaneously or following experimental procedures.

The medial lesions in this case differ considerably from the usual descriptions of spontaneous canine arterial diseases. In the most common of these there occur nodular fibrous intimal thickenings that affect principally the abdominal aorta. Fatty changes in these nodules are uncommon and primary deposits of fat in the otherwise uninvolved vessel wall are practically unknown. Medial calcification histologically similar to Mönckeberg's sclerosis is not unusual and predominantly involves the aortic arch in the vicinity of the aortic valves. Morehead and Little<sup>1</sup> have recently described, in addition, such spontaneous aortic lesions as focal elastic tissue loss, with "grouping" of smooth muscle cells and medial necrosis with cyst formation. Multiple calcium deposits in the vascular walls as well as in the myocardium, endocardium, kidneys, and numerous other organs are often associated with leptospirosis and uremia.<sup>2</sup> Necrotizing endarteritis of the pulmonary artery, aorta, coronaries, and other vessels, sometimes complicated with dystrophic calcification, is commonly observed with uremia due to various causes. Hyalinization of the splenic arterioles is regularly seen in older dogs. Diffuse arterial sclerosis with elastic hyperplasia, rheumatic arteritis, thrombo-angiitis obliterans and endarteritis obliterans are unknown in this animal. A common finding in purulent metritis is a thrombo-arteritis of the pulmonary vessels, and lesions identical with human periarteritis nodosa have been described in dogs.<sup>3</sup>

A survey of the available literature indicates that primary xanthomatosis and secondary xanthomatosis resulting from hyperlipemia have not been noted in any of the tissues of domestic animals although personal observations have demonstrated the presence of foam cells in some neoplasms and inflammatory lesions.

\* Received for publication, July 16, 1945.

## MATERIAL AND METHODS

The reported case was that of a dog brought to my animal hospital for treatment. Euthanasia was performed with soluble pentobarbital given intravenously, followed by immediate necropsy. The tissues were fixed in Zenker's fluid-formalin solution and 10 per cent solution of neutral formaldehyde, U.S.P. Paraffin sections of the former were stained with hematoxylin and eosin, Masson's trichrome stain,<sup>4</sup> Foot's modification of Hortega's silver carbonate method for reticulum,<sup>5</sup> Mallory's phosphotungstic acid hematoxylin, Dominici's stain,<sup>6</sup> Verhoeff's elastica and van Gieson's stains. Formalin-preserved tissue was embedded in gelatin<sup>7</sup> and Lison's<sup>8</sup> tabular method of lipid analysis was applied to frozen sections using sudan IV, Nile blue sulfate, the Lorrain Smith-Dietrich method, and the Schultz and Romieu technics. The von Kossa technic for calcium, and microchemical examination for inorganic and organic iron were performed on frozen sections.<sup>9</sup>

## CLINICAL DATA

The animal was an 8-year-old English setter that had been under my care since 3 months of age. The past history consisted of frequent attacks of gastro-enteritis, the removal of a solitary mast cell tumor (Case 2 of a previous report<sup>10</sup>), and the occurrence of acute interstitial nephritis at the age of 6 years. When 8 years old, the animal was brought to the hospital for anorexia of 6 days' duration, occasional vomiting, weakness, and inability to walk for 3 days. Examination of the urine disclosed a specific gravity of 1.018, a 1 plus albumin test, and occasional hyaline and granular casts. The blood urea nitrogen was 31.0 mg. per cent. A blood count showed 4,880,000 red cells, 8.9 gm. of hemoglobin (Newcomer method), and 22,000 white cells. The differential percentages were: segmented neutrophils, 40; nonsegmented neutrophils, 42; lymphocytes, 5; and monocytes, 13. Bone marrow aspirated from the crest of the ilium by the method previously described<sup>11</sup> indicated: segmented neutrophils, 29.6 per cent; nonsegmented neutrophils, 45.0 per cent; lymphocytes, 6.6 per cent; monoblasts, 0.6 per cent; monocytes, 17.0 per cent; normoblasts, 0.6 per cent; hematogones, 0.2 per cent; and mast cells, 0.8 per cent.

In view of the moribund status of the animal, the owner consented to euthanasia.

## MACROSCOPIC OBSERVATIONS

The gross lesions of the arteries were especially evident in the kidneys, heart, spleen, prostate, liver, lymph nodes, skin, adrenals, and pancreas. In longitudinal and cross sections the arteries were thickened, their walls were prominent and contained numerous, nodular, yellow-tan lesions measuring from 0.1 to 0.2 mm. in diameter (Figs. 1 to 3).

The carotid artery presented a smooth intima with many confluent yellow-tan areas ranging from 0.5 to 2.0 mm. in diameter. The thoracic

and abdominal portions of the aorta contained occasional yellow-tan lesions similar to those in the carotid artery.

The capsules of both kidneys were adherent and the external surfaces nodular. They were uniformly grayish tan and firm to palpation. The striations were obscured.

The auriculoventricular valves were wrinkled and nodular and the semilunar valves were normal. The left ventricle was thickened. Small, irregular, gray areas of fibrosis were seen in the cardiac muscle.

The prostate was approximately twice its normal size. The cut surface was slightly nodular and sections showed lobulation resulting from fibrous trabecular proliferation with numerous small and large cyst-like structures.

The other organs were normal grossly.

#### MICROSCOPIC OBSERVATIONS

##### *The Lesions in the Muscular Arteries*

A survey of the different organs revealed similar arterial alterations and, although the atheroma-like process varied in degree and extent in the different vessels, a logical sequence in the development of the pathologic process could be readily established. The vascular involvement was conspicuous in the kidneys, heart, prostate, spleen, liver, skin, lymph nodes, adrenals, and pancreas, with few affected vessels in the remaining organs. The veins were entirely normal. The aorta and carotid artery showed changes similar to those of the muscular arteries (Fig. 8).

As seen in paraffin sections from which fat had been removed, the earliest lesion consisted of groups of foam cells located in the media (Fig. 4). The foam cells varied from 8 to 35  $\mu$  in diameter and were round, oval, or polyhedral. They were closely packed, but a distinct cell membrane clearly outlined each cell. The cytoplasm presented a fine, mesh-like appearance and consisted of delicate strands forming minute round or oval spaces. Many cells contained in addition one or several large cytoplasmic vacuoles. The nuclear structure was vesicular with a few fine chromatin granules. The nuclei were round, oval, irregular, elongated and frequently crenated. Each cell contained usually one, sometimes two, and occasionally three or four nuclei that were centrally or peripherally located. The majority of the nuclei had one or two small central or eccentric nucleoli.

In frozen sections, the lipids in the foam cells existed as small, spherical, closely packed but discrete droplets, and as large, round, oval, or irregular drops and masses (Figs. 10 and 11). Both forms sometimes occurred in the same cell. The fat was largely intracellular,

although the larger droplets and masses were occasionally extracellular. Fine droplets were also present in muscle cells immediately adjacent to the foam cells.

Histochemical studies of the lipids in the foam cells, following the interpretations formulated by Lison,<sup>8</sup> revealed the following data. The lipids were a dirty grayish brown in unstained frozen sections. Treatment with Lugol's solution produced no black-green or brown color indicating the absence of carotinoids; sulfuric acid caused no red color, suggesting the absence of chromolipoids. With the Schultz test the lipids gave a rich blue-green color, and with the Romieu test the initial reddish purple coloration gradually turned green. Both of these modifications of the Liebermann-Burchard reaction are specific for cholesterol and its esters. Mounted unstained frozen sections were doubly refractive under crossed Nicol prisms but showed no cross of polarization (Fig. 9). When the slide was heated, the anisotropic material greatly decreased in amount. With sudan IV the lipids stained a deep red-orange (Fig. 10). The Lorrain Smith-Dietrich method produced a dark blue coloration which is characteristic for a lipin only if cholesterol is absent. Nile blue sulfate produced color ranges from rose to dark blue, with intergrading variations such as lilac, purple, purple-red, and purple-blue occurring in many instances in the same artery (Fig. 11). The rose color signifies the presence of nonsaturated glycerides whereas the blue color is nonspecific.

The collections of lipid-filled cells assumed a nodular form and the larger groups increased the width of the media from two to three times (Fig. 4). They were located either in the external or middle media, and, when in the former, the adventitia was sometimes thinned. The xanthoma-like masses contained only rare argyrophilic and thin connective tissue fibers; elastic fibrils and muscle cells had disappeared. At the periphery of the foam cell conglomerates were seen muscle cells with minute fat droplets in their cytoplasm. These muscle cells showed the normal type of elongated muscle nucleus with few fine chromatin granules and a small nucleolus. Other muscle cells with increased lipid content contained a plumper, more rounded nucleus similar to that of the foam cells lying near them. There were many cells of intermediate form so that numerous transitional stages between normal muscle cells without lipid and the foam cells were present. Usually a normal zone of media existed between the xanthomatous lesion and the intact internal elastica. The adjacent media and the intima underlying the foam cells were normal. This early stage was focal, evinced no inflammatory reactions or fibrosis, and was confined to the media.

In addition to the small focal xanthomatous formations that occurred principally in the larger arteries, numerous smaller arteries showed either diffuse eccentric or concentric replacement of their media with collections of foam cells, so that the width of the media was increased from five to fifteen times (Fig. 5). In these vessels, the inner elastica was usually intact and the intima and adventitia were normal.

Frequently the medial lipidosis extended up to and included the intima (Fig. 6). The internal elastica usually abruptly disappeared at this point and intimal atheromas, somewhat similar to those in man and those experimentally produced in rabbits by cholesterol feeding, sometimes appeared. No intimal atheromas were found, however, without previous involvement of the media. In occasional arteries, small collections of red cells were interspersed between the foam cells.

The next stage observed in the development of the medial lesion was the proliferation of fine connective tissue fibrils and strands in the areas of fatty deposit. These fibrils gradually became coarser and denser (Figs. 4, 6, and 7). The fibrosis was irregular and cellular in the early stages. The connective tissue proliferation partially, but only rarely completely, replaced the foam cells. Frequently argyrophilic fibers and dense fibrous strands encircled individual foam cells. Inflammatory and giant cells were absent, and in the scarred areas muscle and elastic tissue had largely disappeared. The fibrosis was usually associated with arteriolar proliferation even in areas with minimal connective tissue formation.

Occurring simultaneously with the fibrous proliferation, and to a lesser degree in arteries without fibrosis, patchy areas were found infiltrated with material giving the microchemical reactions of iron (Fig. 12). At first, these masses, that stained deep blue with hematoxylin, were thought to be calcium, but von Kossa's stain was negative. In unstained frozen sections the iron deposits were a pale lemon-yellow that contrasted with the yellow-brown of hemosiderin in the spleen. Treatment with potassium ferrocyanide and potassium ferricyanide produced no coloration, indicating the absence of ferric and ferrous salts of inorganic iron. A positive Prussian blue reaction followed exposure to hydrochloric acid and potassium ferrocyanide, proving the presence of organic iron. The iron-containing material existed as small and large round granules, irregular large clumps and masses, and occasionally as crystalline, sheet-like plates. The iron was usually extracellular although small spherules occurred in some foam cells and adventitial histiocytes. Considerable quantitative variation

was found in the different arteries, the iron being absent in some and almost completely filling others.

The accumulative deposit of lipids, connective tissue, and iron was progressive so that the different arteries showed various stages of the pathologic process, transitions often being observed in the same artery. Eventually many arteries showed an irregular concentric distribution affecting all coats, with thickening of the adventitia, and fibrosis and lipidosis extending throughout the media and intima with only occasional strands of the inner elastica persisting (Fig. 7). The vessel lumina were narrowed and completely obliterated in some instances and occasionally filled with foam cells. The pathologic process, therefore, eventually completely transformed the entire structure of the artery (metallaxis).

#### *The Lesions in the Parenchymal Organs*

As has been previously stated, the arterial lesions were similar in all organs examined. In addition, various organic alterations were observed, commonly encountered in older dogs and unrelated to the vascular changes.

##### *Kidneys*

The renal lesion consisted of subacute interstitial nephritis and was of the usual type seen in dogs.<sup>12</sup> There were areas of connective tissue proliferation with interstitial infiltrations of lymphocytic cells. The glomeruli showed various changes consisting of distortion of the tufts, fibrosis, and periglomerular fibrosis, but many were normal. Numerous collecting tubules in the medulla were greatly dilated and hyperplastic. Hyperplastic proximal convolutions, in addition to dilated tubules with flattened epithelium, occurred in the cortex.

Besides the arterial lesions described above, lipid stains revealed that the lumina of the interstitial capillaries and of many arteries and veins contained an amorphous material that was pale yellow-orange with Sudan IV, pale purple with Nile blue sulfate, pale blue-black with the Lorain Smith-Dietrich method, gave a positive Liebermann-Burchard reaction, and was doubly refractive with the polarizing microscope. Similar material occurred in the glomerular capillaries and in some Bowman's spaces. Minute fat droplets were frequently present in the glomerular tufts. The epithelial cells of many collecting tubules and some hyperplastic collecting tubules contained numerous fat droplets. The cells of the remainder of the nephron occasionally had fat droplets in their cytoplasm. The physiologic fat-rich terminal portion of the proximal convolution, however, showed a striking reduction in the lipid content of the epithelial cells, a finding not unusual in canine interstitial nephritis. In the interstitial tissue a moderate num-

ber of connective tissue cells and macrophages contained fine fat droplets. Iron-staining material in the form of granules and small clumps occurred in some interstitial macrophages.

#### Prostate

Large areas of cystadenomatous hyperplasia associated in some regions with fibrosis and round-cell infiltrations alternated with relatively normal areas of prostatic tissue. These changes are commonly seen in old dogs.

With lipid stains the lumina of many cystically dilated acini and of a few normal acini contained an amorphous material similar in staining reactions to the amorphous material in the vascular lumina. In addition, numerous small and large fat droplets were dispersed throughout this material that gave identical but deeper staining reactions. Fat droplets staining like the arterial lipids occurred in some epithelial cells of the glandular tissue, macrophages, fibroblasts, and muscle cells. A few iron granules occurred in occasional fat-filled histiocytes and in the amorphous lipid-staining material of the glandular lumina.

#### Spleen

The general splenic structure was maintained and the changes were those usually seen in older dogs. There were increased numbers of megakaryocytes, a moderate amount of hemosiderin that was principally intracellular, a mild degree of atrophy, focal congestion of some sinuses, and few lymph nodules. The central arterioles showed the hyalinization that is characteristic in dogs over 5 years of age.

In paraffin sections foam cells were absent in the hyalinized arterioles but fat stains indicated ample quantities of small and large lipid droplets, that stained as did the lipids in the muscular arteries. Fat droplets were present in some histiocytes, often associated with hemosiderin. The vessel lumina contained material similar to that seen in other organs but amorphous lipid-staining material rarely occurred in the venous sinuses. The arteries within the spleen were involved with siderosis more frequently than those of other organs. The fatty hyalinized arterioles, on the other hand, rarely showed iron deposition.

#### Liver

The liver was normal with the exception of congestion of some sinusoids, hemosiderosis of many Kupffer cells, and occasional vacuolization of hepatic cells.

In addition to the lipid amorphous material in the vascular lumina, similar material occurred in some sinusoids and perivascular lym-

phatics. The bile duct epithelium contained many lipid droplets. Discrete fat droplets of the physiologic type were present in the hepatic cells. These stained orange-red with Sudan IV, blue with Nile blue sulfate, blue-black with the Lorraine Smith-Dietrich method, and were negative to the Liebermann-Burchard reaction. The Kupffer cells contained relatively few fat droplets that tinctorially resembled the lipids in the foam cells. Iron-staining material occurred only in the Kupffer cells.

#### Other Organs

The remaining organs, with the exception of mild testicular atrophy, pulmonary edema, hypoplastic bone marrow, fibrosed auriculoventricular valves, and focal areas of myocardial fibrosis, were normal. The vascular lumina of all organs, however, contained amorphous lipid-staining material identical with that in the renal vessels.

#### COMMENT

The combination of pathologic processes here described, consisting of a deposit of fat in foam cells in the arterial media with a subsequent fibrosis and siderosis that progressively affected all coats of the arteries, can be suitably summarized as athero-fibro-siderosis. It is unnecessary to discuss at length the experimental arterial lesions that have been produced by various means since comparable changes have not been induced. Similar spontaneous vascular lesions are unknown in any other species, including man. This widespread medial lipidosis of the muscular arteries therefore is unusual, for fat deposition in human pathology is much less frequent in muscular than in elastic arteries and medial fat is rare except as an extension of atheromatous foci in the intima.<sup>13</sup> In the rare arterial involvement of human xanthomatosis, the intima is also the site of lipidosis.

The arterial lesions can be interpreted either as a peculiar and unusual type of atherosclerosis with medial localization of the fatty deposits or as a vascular manifestation of that disturbance of lipid metabolism known as xanthomatosis. The latter concept appears more plausible in view of the histologic appearance of the lesion. Although the general histologic picture of the canine vascular involvement resembled the xanthomatous lesions in man, certain differences existed. In the latter, in addition to foam cells and fibrosis, inflammatory cells, Touton giant cells, plasma cells, and eosinophils may be present. These cellular elements were all conspicuously absent in the dog.

Thannhauser<sup>14</sup> has classified the xanthomatous diseases into primary essential xanthomatosis, secondary xanthomatosis due to hyperlipemia, and localized xanthoma formation in inflammatory tissue and

true tumors. The secondary xanthomatosis due to hyperlipemia occurs in diabetes mellitus, chronic pancreatitis, glycogen storage disease, and lipoid nephrosis. These conditions were absent in this animal, and, furthermore, the microscopic lesions of human secondary xanthomatosis resulting from hyperlipemia differ considerably from the arterial changes here described. Beside the fact that the arteries alone were affected in this case, in human hyperlipemia foam cells are sparse and extracellular fat deposits occur in the inflammatory connective tissue. That the arterial lipidosis exemplified local xanthoma formation in inflammatory tissue can be discounted from the histologic appearances since the deposit preceded the tissue reaction. The evidence suggests, therefore, that the arterial lesions can be classified with those belonging to the group of primary essential xanthomatoses. In the latter the blood cholesterol may be either normal or elevated, and it is of interest that in man, as in this case, the vascular lesions are usually associated with hypercholesterolemia.

Different theories exist as to the mechanism of xanthoma formation. Pick and Pinkus<sup>15</sup> suggested that the hypercholesterolemia led to an increase of this substance as well as other fats in the cells. Aschoff<sup>16</sup> believed that the reticulo-endothelial system took up and retained the lipids from the blood stream. Bloch,<sup>17</sup> Schaaf,<sup>18</sup> and Schaaf and Werner<sup>19</sup> considered an extracellular general metabolic disturbance of lipids at fault and that "oids are secondarily deposited in the reticular cells and tissue. Thannhauser and Magendantz<sup>20</sup> distinguished etiologically between essential and secondary xanthomatosis. They considered essential xanthomatosis a systemic disorder of the intracellular metabolism, and secondary xanthomatosis the result of cholesterol infiltration and deposition from hyperlipemic serum.

The relationship of the hypercholesterolemia to the vascular lesions in this case requires further comment. It is well known that, contrary to results in rabbits, it is difficult to induce hypercholesterolemia in dogs. However, elevation of the blood cholesterol occurs normally in carnivores during pregnancy and following castration.<sup>21</sup> In addition, persistent hypercholesterolemia has been experimentally produced in these animals but atheromatosis has never been observed.<sup>21</sup> Cholesterol feeding to dogs results in the deposition of neutral fats and cholesterol in hepatic cells,<sup>22</sup> while in this instance the liver fat gave a negative Liebermann-Burchard reaction. In spontaneous canine diabetes mellitus<sup>23</sup> and obstructive jaundice,<sup>24</sup> the blood cholesterol is increased but atheromatous vascular lesions are absent. This evidence therefore casts doubt on the assumption that the hypercholesterolemia which was present in this case led to the arterial lipidosis.

Different opinions have been expressed concerning the derivation of the foam cells. Their origin has been summarized by Gruenfeld and Seelig.<sup>25</sup> Spindle cells of hypertrophied connective tissue, fibroblasts, endothelial cells, degeneration of muscle cells in xanthoma of the eyelids, Kupffer cells, reticulum and periadventitial cells, cells of pulmonary alveoli, glandular epithelium, histiocytes, nerve cells, and the cells of numerous tumors have been considered. Differentiation, of course, must be made between primary and secondary xanthomatosis with respect to the progenitors of the foam cells.

The histogenesis of the arterial lesions in this instance can be traced to the early xanthoma-like masses in which transitional forms between normal muscle cells and foam cells were observed. The concept of muscle transformation into lipid cells gains support from the fact that the media of muscular arteries consists almost exclusively of smooth muscle cells with thin reticular fiber membranes and thin elastic networks.<sup>26</sup> In addition, transitional stages indicating the formation of foam cells from the occasional subendothelial macrophages and adventitial histiocytes were not seen. The endothelial cells can be eliminated, since in the early stages fat droplets were absent from them and the process was deep in the media. Nor is it likely that the rare fibroblasts of the subendothelial region or media took part in the formation of foam cells because these cells appeared in large numbers only later in the process as part of the tissue reaction and did not contain fat. The possibility of mononuclear cells derived from the blood appearing as small lymphocytic and monocytic cells and serving as the parent cell of the xanthomatous tissue, as suggested by Anitschkow<sup>27</sup> in experimental atherosclerosis in the rabbit, is negated by the absence of similar cells in the described lesions.

A consideration of the nature of the fats seen in the foam cells and medial deposits becomes involved in the difficulties inherent in the interpretation of fat stains for the identification of lipids. These difficulties have been demonstrated by Arndt,<sup>28</sup> Kaufmann and Lehmann,<sup>29</sup> Lison,<sup>8</sup> and others. My histochemical studies followed the interpretations of Lison,<sup>8</sup> and indicated that the arterial fats in this case consist of nonsaturated glycerides and cholesterol or its esters. In man the lipids in xanthomas and atheromatous deposits are also mixtures of fatty substances<sup>14, 30, 31</sup> while in the canine nodular intimal aortic plaques the fat, when present, is neutral.<sup>8</sup>

Collection of iron-containing material is also an unusual lesion in arteries. The siderosis of the vascular walls of the globus pallidus and the brown, iron-staining pigment in the thickened intima of small and medium-sized arteries in pulmonary sidero-silicosis in man are dif-

ferent from the lesion in this dog in their pathogenesis and histology. In human arteriosclerosis, iron is often found in association with calcification<sup>30, 32, 33</sup> but no mention is made in the literature of the presence of iron alone in arteriosclerosis or in any experimental vascular lesion. In human xanthomatous lesions, hemosiderin and red cells are not uncommon. Iron pigment and erythrocytes may also occur in Gaucher cells but are usually absent in Niemann-Pick cells.<sup>34</sup> The iron-containing material in this instance was apparently not identical with hemosiderin, as the latter did not stain with hematoxylin but remained a golden yellow while the iron-containing material stained deep blue. The source of the iron-staining material is problematical in the absence of sufficient hemorrhage with consequent disintegration of red cells in the xanthoma masses to explain the widespread siderosis.

#### SUMMARY

An 8-year-old dog showed at necropsy vascular lesions that have not been described as occurring spontaneously or following experimental procedures. These can be classified as a primary essential xanthomatosis of the hypercholesteremic type. Initially, foam cells containing nonsaturated glycerides and cholesterol or its esters appeared in the media of muscular arteries and to a lesser degree in the elastic arteries. The histogenetic evidence indicates that the lipid-containing cells were derived from the smooth muscle cells of the media. Subsequently, connective tissue proliferation developed in which arteriolar formation was prominent. Iron-staining material that differed tinctorially from hemosiderin occurred in the majority of arteries involved in the xanthomatosis. Eventually, in many vessels the entire structure was transformed (metallaxis) by the combination of atheromatosis, fibrosis, and siderosis.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

### PLATE 109

FIG. 1. Section of kidney showing the prominent thickened arteries in the cortex. Approximately  $\times \frac{3}{4}$ .

FIG. 2. Section of hypertrophied left ventricle with prominent arteries. Approximately  $\times \frac{3}{4}$ .

FIG. 3. External surface of portion of heart muscle showing the thickened coronaries, the walls of which contained small nodular lesions. Approximately  $\times \frac{3}{4}$ .

FIG. 4. Longitudinal section of renal artery showing an early xanthoma nodule in the upper left segment of the vessel. The foam cells extend up to, but do not involve the adventitia. The internal elastica is normal and the adjacent media evidences no changes. The lower right segment of the same artery illustrates more advanced changes in the lesion. The inner elastica has disappeared and foam cells can be seen in the intimal region. The xanthoma mass has increased in size and there is partial replacement with fibrous connective tissue, although many foam cells are encircled by connective tissue fibers. Verhoeff's elastica and van Gieson's stains.  $\times 100$ .

FIG. 5. Cross section of a smaller renal artery than that shown in Figure 4 with eccentric thickening of the media by foam cells. The internal elastica is normal and foam cells are absent in the intima. This is an early stage in the lesion and fibrosis is absent. Verhoeff's elastica and van Gieson's stains.  $\times 120$ .

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Bloom

Xanthomatosis of the Arterial Media

PLATE 110

FIG. 6. Cross section of a small renal artery demonstrating a later developmental stage of the medial lesion. Above, a small segment of the artery is normal and, although the inner elastica persists in several areas, the media shows replacement with foam cells with a moderate proliferation of connective tissue. In the lower left segment, foam cells are present in the thickened adventitia. The lumen contains foam cells. Verhoeff's elastica and van Gieson's stains.  $\times 150$ .

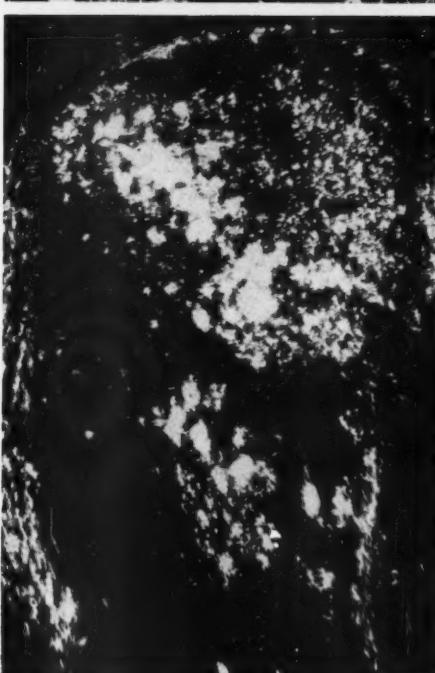
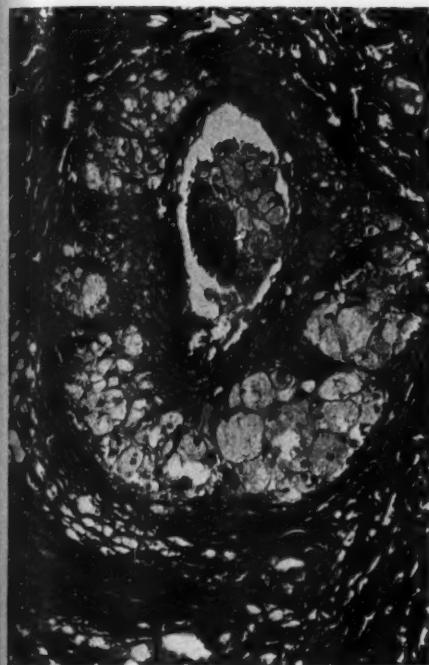
FIG. 7. Cross section of a renal artery with foam cells, connective tissue, and iron-staining material. The media is greatly thickened and the upper portion of the vessel shows fibrosis in which there are arterioles alternating with foam cells. The dark-staining granules in the media are iron-containing material. The elastica has disappeared and the normal arterial structure is completely transformed. Verhoeff's elastica and van Gieson's stains.  $\times 100$ .

FIG. 8. Cross section of portion of carotid artery. Above, the artery is normal. Immediately adjacent there is foam-cell formation in the media with disappearance of many elastic fibers and moderate fibrosis. Verhoeff's elastica and van Gieson's stains.  $\times 65$ .

FIG. 9. Frozen section of the same renal artery as in Figures 10 to 12, showing the large amount of anisotropic lipids seen with the polarizing microscope.  $\times 150$ .







Bloom

Xanthomatosis of the Arterial Media

PLATE III

FIG. 10. Adjacent section to that shown as Figure 9, illustrating the lipids in the foam cells. The lipid distribution is identical with that in the preceding figure. The amorphous deep-staining masses in the right portion of the photomicrograph are iron-containing material. Sudan IV and hematoxylin stains.  $\times 150$ .

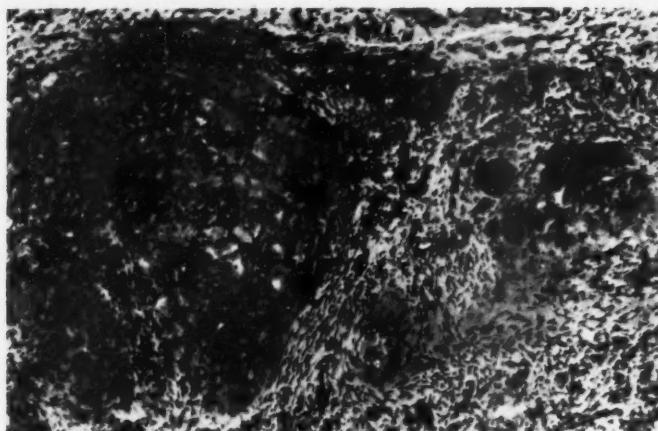
FIG. 11. Serial section of the same artery as in Figure 9, stained with Nile blue sulfate. The lipid distribution is identical with that in the two preceding figures. The rose-colored lipids signify the presence of nonsaturated glycerides, whereas the blue-stained fat is nonspecific.  $\times 150$ .

FIG. 12. Serial section of the same artery as in Figure 9, demonstrating a positive Prussian blue reaction of the amorphous, deep-staining material in the right portion of Figure 10. Counterstained with carmine.  $\times 150$ .

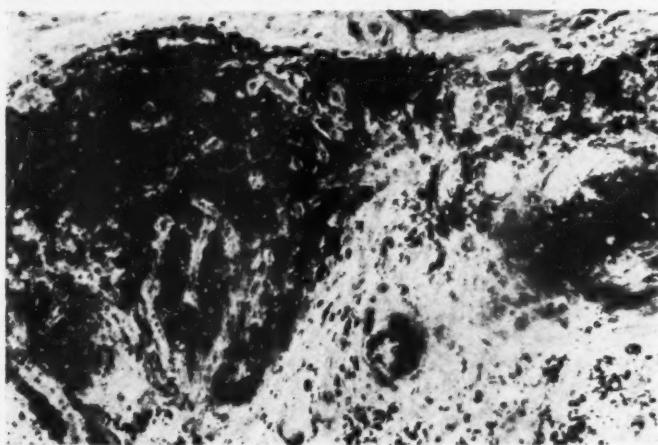




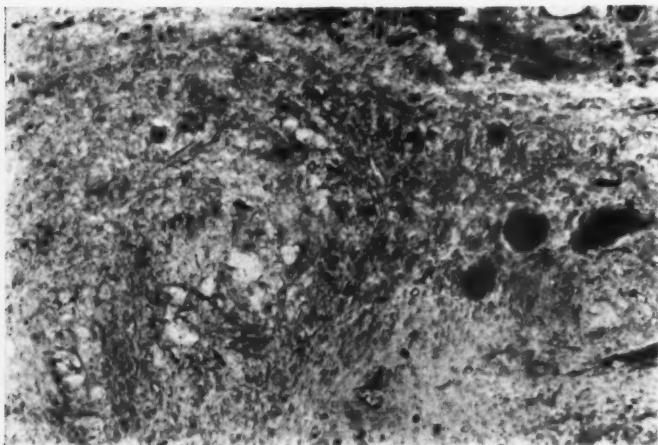
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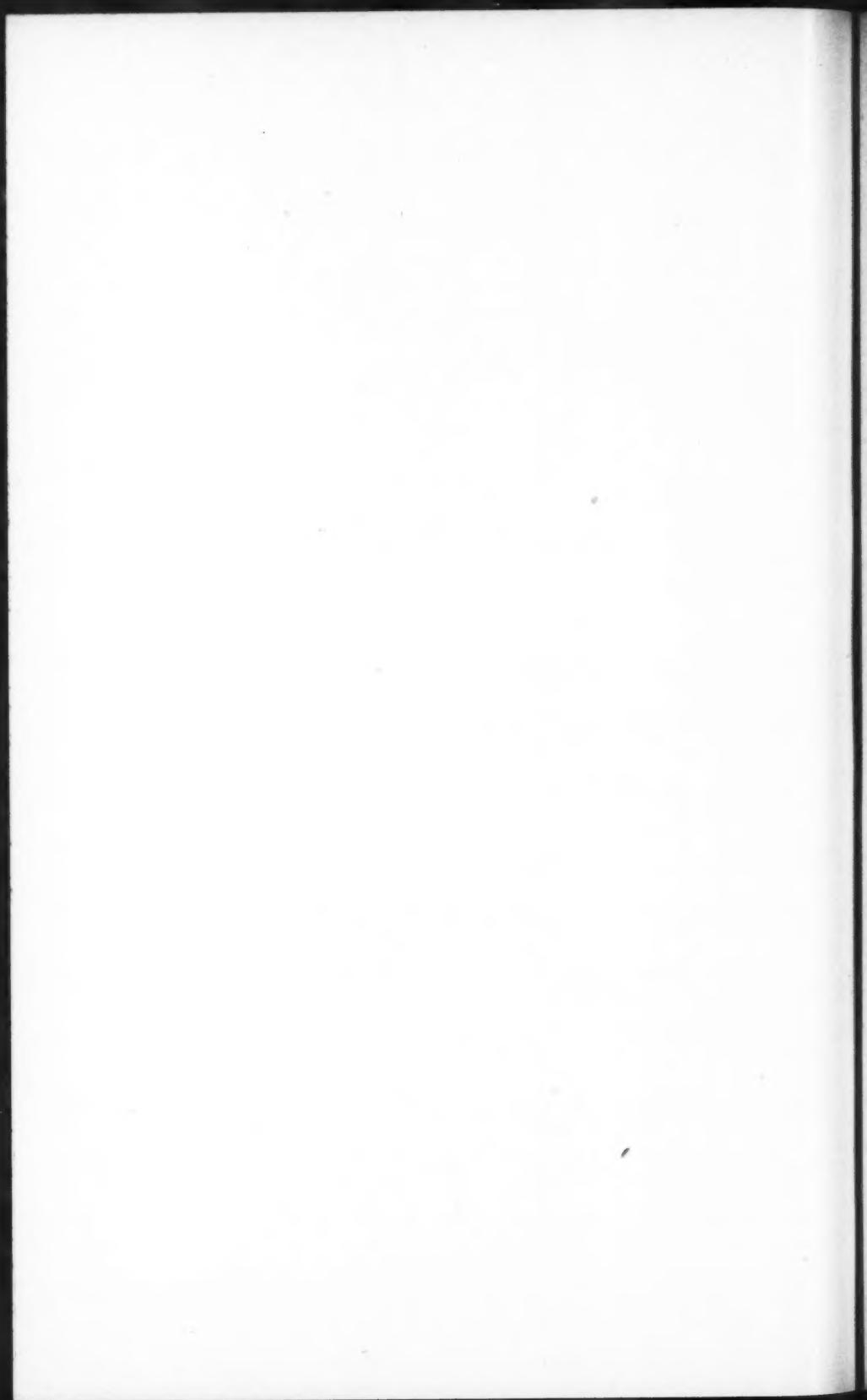


2



Bloom

Xanthomatosis of the Arterial Media



## MALIGNANT GRANULOSA CELL TUMOR WITH PSEUDOTUBERCLES \*

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Granulosa cell tumors of the ovary are being reported in increasing numbers. They constituted 2.11 per cent of 1,728 ovarian tumors, according to statistical studies compiled by Szathmáry,<sup>1</sup> Klaften,<sup>2</sup> and Fauvet.<sup>3</sup> Thornton<sup>4</sup> stated that "granulosa cell tumors constitute 8 to 10 per cent of all ovarian carcinomas."

Granulosa cell tumors may be encountered at any age. Holmes and Hauck<sup>5</sup> reported 60 per cent occurring after the menopause, 30 per cent during the child-bearing period, and 10 per cent before puberty. In this connection it is interesting to note the report of a case by Banks<sup>6</sup> in an infant 17 months of age.

The majority of granulosa cell tumors are considered benign, only 5 to 10 per cent being malignant, according to Te Linde.<sup>7</sup> Recent reports of malignant forms are those of Norris,<sup>8</sup> McCartney,<sup>9</sup> Henderson,<sup>10</sup> and Harris.<sup>11</sup> In these reports, except that of Norris, the criteria of malignancy were recurrences or metastases. In Norris' case there were numerous mitotic figures and the tumor was of diffuse type. According to Schiller<sup>12</sup> such histological features, however, are usually not considered as entirely reliable for the demonstration of malignancy in a granulosa cell tumor. The only other criterion of malignancy sometimes given is that stated by Pratt,<sup>13</sup> according to whom these tumors are more often bilateral when malignant. In Klaften's series,<sup>2</sup> 6.2 per cent were bilateral. Dockerty and MacCarty<sup>14</sup> stated that 90 per cent were unilateral.

Conflicting views are expressed as to the histogenesis of granulosa cell tumors. It was formerly accepted that granulosa cells originated from the celomic epithelium. Morehead and Bowman,<sup>15</sup> in a recent article, took issue with this theory of origin and contended that the granulosa cells are derived from the mesenchyme. If this assumption is correct, it would explain the extra-ovarian location and occurrence of granulosa cell tumors of the uterus, broad ligament, and retroperitoneum.

The well-recognized histological types are folliculoid, cylindromatous, and diffuse. The presence of Call-Exner bodies in these tumors is usually considered diagnostic. Another feature which aids in the diagnosis is evidence of estrogenic hormonal activity as reflected by

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the proliferative "Swiss-cheese" type of endometrium. According to Wolfe and Kaminester,<sup>16</sup> however, secretion by these tumors may be lacking, especially in those which are immature and rapidly growing and show a diffuse or medullary pattern. On the other hand, estrogen production may be so marked as to be considered the carcinogenic agent for a malignant neoplasm of the endometrium by its influence, according to Stohr.<sup>17</sup> Dockerty and MacCarty<sup>18</sup> stressed the low degree of malignancy of granulosa cell tumors and discussed their radiosensitivity. The association of adenocarcinoma of the uterus with a granulosa cell tumor of the ovary was reported. These authors also emphasized the importance of excess theelin as a carcinogenic agent. An increasing number of carcinomas of the endometrium are being reported in connection with granulosa and theca cell tumors of the ovary. Recently reported cases are those of Ingraham, Black, and Rutledge,<sup>19</sup> three cases, and Stohr,<sup>17</sup> three cases. It is doubtful whether adenocarcinoma of the uterus can be explained on the carcinogenic action of theelin alone. The extensive endometrial hyperplasia, with the glandular elements remaining in the "persistent proliferative phase" over a long period of time, resulting from excess estrin production, no doubt plays an important rôle. The importance of suspecting granulosa cell tumor in any patient past the menopause complaining of vaginal bleeding, especially when curettage reveals endometrial hyperplasia, should be stressed.

An unusual and interesting report of a case is that of Murray, Dockerty, and Pemberton,<sup>20</sup> who discussed the coexistence of granulosa cell tumor and ovarian teratoma containing thyroid tissue.

We have recently encountered a case in which there were bilateral tumors of the ovaries with many mitotic figures and well defined Call-Exner bodies. Metastases to the omentum and extension to the fallopian tubes and uterus were also present. In addition, definite pseudotubercles were found histologically in these tumors. Aside from the mention of pseudotubercles by Ewing<sup>21</sup> and the description of giant cells in a metastasis in the brain by McCartney,<sup>9</sup> this feature has not been emphasized in connection with granulosa cell tumors. Because of the peculiar and unusual histopathological structure which might lead to confusion in diagnosis, it was felt that a report was warranted.

#### REPORT OF CASE

The patient was a colored female, 38 years of age, who was admitted to the hospital on January 26, 1942, complaining of a "pressing down feeling" in her abdomen. Her abdomen had been becoming more and more protuberant since April, 1941. During this time there was gradually increasing lower abdominal

discomfort. She was having a scanty vaginal discharge of blood. Her preceding menstrual period was of 3 days' duration during the second week of December, 1941. The flow at that time was scanty. Before December, 1941, menses occurred every 28 days, lasting for 3 or 4 days, with no indication of menorrhagia, or dysmenorrhea. She was gravida 12, and para 9; she had five living children.

On examination, the temperature was 98.6° F.; pulse, 80; and respiration, 20 per minute. The patient was poorly nourished, though well developed. A small subcutaneous nodule was noted in the left hypochondrium. The thyroid gland was symmetrically enlarged; no signs or symptoms of toxicity were manifested, however. The abdomen was markedly prominent and without tenderness or rigidity. A smooth, round, freely movable mass was palpated in the lower abdomen. On vaginal examination, cystocele, rectocele, and chronic cervicitis were noted. The uterus was anterior, but displaced to the left. The left cornu of the uterus was attached to, or continuous with, a large mass posterior and to the left of the uterus, extending downward into the cul-de-sac. This mass was firm and could not be completely displaced out of the pelvis. It was described as being about the size of a uterus with 4½ to 5 months' pregnancy.

On February 5, 1942, a laparotomy was performed. Large tumors of both ovaries were encountered. There was also a yellowish brown, firm nodule, measuring 0.5 cm. in diameter, in the omentum. Bilateral salpingo-oophorectomy, hysterectomy, and excision of the omental mass were performed.

Postoperatively, the patient developed abdominal distention, nausea, and vomiting. Her course was a stormy one, but she recovered satisfactorily and was discharged on the 40th postoperative day.

#### *Gross Findings*

The specimens submitted for examination were a portion of omentum, a uterus, two fallopian tubes, and two ovarian tumor masses. The portion of omentum measured 6.5 by 1.5 cm. It was thin and pale gray, with a few small, yellow, granular areas and a larger yellowish brown, firm nodule measuring 0.5 cm. in diameter. The uterus and cervix together measured 10 by 6 by 4 cm. Just above the internal os, the endometrium was slightly roughened and granular. On the postero-superior surface of the uterus there was an elevated area, 2.5 cm. in diameter, which was soft and yellowish brown. It appeared to extend for a short distance into the myometrium. The serosal surface of the right fallopian tube was smooth except distally where it was granular and yellowish brown. The left fallopian tube showed no tumor implantation on its surface. The two tumor masses with portions of the ovaries were separate from the uterus. The larger one measured 18 by 15 by 8 cm. On its superior surface a portion of fallopian tube was present. The external surface of this tumor mass was lobulated (Fig. 1). Numerous areas of hemorrhage were noted. The rest of the tumor was yellowish brown. At the lower pole, a thick-walled cavity, measuring 5.5 cm. in diameter, was noted. The inner surface was smooth and pale and an area of necrosis, 2 cm. in diameter, formed part of its boundary. The tumor, in general, was soft and mushy. The cut sur-

face was yellowish brown and appeared nodular. The other tube and ovary made up a small mass measuring 11.5 by 8.5 by 6 cm. This mass was very soft and had a lobulated external surface showing several large cysts varying in size from 2.5 cm. to 4.5 cm. in diameter. The largest of these was filled with a serohemorrhagic fluid. The cysts were thin-walled and transparent. The portion of fallopian tube attached to this mass showed some small nodules on its external surface. The tumor proper was light yellowish brown, nodular, and edematous, with only small areas of necrosis.

#### *Microscopical Findings*

On histopathological examination, the ovarian tumors were found to be made up largely of diffusely arranged cells with round, dark nuclei surrounded by very small amounts of cytoplasm. In some areas these cells were quite closely packed, while in others they were widely separated. Here and there, rather well defined follicular structures with ovum-like bodies in their centers (Call-Exner bodies) were seen (Fig. 2). Much degeneration and necrosis were present. Scattered throughout the sections were numerous pseudotubercles made up of epithelioid cells and some giant cells, and occasionally showing small central areas of necrosis (Fig. 3). The giant cells contained many nuclei arranged at the periphery in some and in the center in others (Fig. 4). Lymphoid cells were not seen. The tumor cells showed mitotic figures in moderate numbers. In one area, lutein cells were arranged in a cyst wall. The cyst contained pink-staining, homogeneous material.

Sections of the fallopian tubes showed extensive invasion with tumor cells. In most areas the tubal mucosa was intact, with the neoplasm destroying the muscularis and serosa. Invasion of uterine musculature and endometrium was also noted. In those areas where the endometrium was not invaded it was thickened and contained straight tubular glands compatible with the persistent proliferative phase (Fig. 5). While some of these glands appeared dilated, no well defined cystic structures suggestive of a "Swiss-cheese" pattern were seen.

The omentum also showed collections of tumor cells which were similar in all respects to those in the ovaries, including the presence of Call-Exner bodies.

*Pathological Diagnoses.* (1) Granulosa cell carcinoma of ovaries (bilateral) with seeding of peritoneum and invasion of tubes and uterus; (2) metastatic granulosa cell carcinoma of omentum.

## DISCUSSION

The diagnosis of granulosa cell tumor in this case is based on the finding of numerous typical Call-Exner bodies among otherwise fairly typical granulosa cells arranged in a diffuse pattern. Endocrine activity was indicated by a persistent proliferative phase in the endometrium. It is realized that "granulosa-cell-like" groups have been reported in dysgerminomas. Novak and Gray<sup>22</sup> have called attention to the tumors which Kermauner and Nürnberg<sup>23</sup> called granulosa cell carcinomas associated with lesions suggestive of tuberculosis. Schiller,<sup>24</sup> in subsequent examination of these tumors, considered them to be dysgerminomas. Nevertheless, it is felt that the granulosa cell masses in the present case are sufficiently definite and extensive to warrant the diagnosis of granulosa cell tumor rather than dysgerminoma. Opinion that the tumor in this case is malignant rests on its seeding of fallopian tubes and uterus, the invasion of the uterine musculature, the fact that it is bilateral and, of lesser importance, the appearance histologically of numerous mitotic figures.

The particular feature of interest here is the presence of pseudotubercles in a granulosa cell tumor. As has been stated, Ewing<sup>21</sup> has called attention to this occurrence, and McCartney<sup>9</sup> has described giant cells in a metastasis from a granulosa cell tumor of the ovary. Of the so-called "special" ovarian tumors, however, the one more usually associated with pseudotubercles is the dysgerminoma. This feature has been pointed out by Ewing,<sup>21</sup> Novak and Gray,<sup>22</sup> Schiller,<sup>24</sup> and Sailer.<sup>25</sup> Seagar,<sup>26</sup> however, in a rather complete discussion of dysgerminomas, did not describe it.

Sailer<sup>25</sup> discussed the presence of pseudotubercles in dysgerminomas at some length. According to him, several explanations have been offered for their presence. Much of his discussion may also apply to the case of granulosa cell tumors.

Some authors have found an associated adnexal or intestinal tuberculosis in these cases. Therefore they have considered the collections of epithelioid cells, fibroblasts, and giant cells as tuberculous. The possibility of an associated tuberculosis in these cases is an interesting question. Aside from the histological features (pseudotubercles), one is reminded of the syndrome of fibroma of the ovary with ascites and hydrothorax, described by Meigs.<sup>27</sup> Repeated efforts have failed to produce evidence of tuberculous lesions or tubercle bacilli in cases showing this syndrome. In this connection the case of Vogt<sup>28</sup> is interesting. He reported a granulosa cell tumor of the ovary associated with hemoperitoneum and hemothorax. The serosanguineous fluid

disappeared from the chest and abdomen 15 days after removal of the ovarian tumor and had not recurred 2 years later. Others have not found tuberculous lesions elsewhere, nor have they demonstrated tubercle bacilli in the lesions in the ovaries.

The most generally accepted explanation is that these pseudotubercles represent a stromal reaction to disintegrating tumor cells. According to Novak and Gray,<sup>22</sup> on the basis of recent laboratory investigations of tuberculosis suggesting that the characteristic tissue reaction is probably produced by fatty substances of fluid nature, it is not unlikely that similar lipids may result from the degeneration of dysgerminoma cells. Since this change may occur in dysgerminomas, it is plausible that a similar finding should be encountered in granulosa cell tumors which also contain lipids. Greenblatt, Greenhill, and Brown<sup>23</sup> have investigated the lipid content of certain ovarian tumors. They found that in such tumors fat may be encountered due to degenerative processes, or as a product of tissue metabolism (hormone storage). The fat in dysgerminomas is believed to be degenerative as it is more abundant in necrotic areas of the tumors. In granulosa cell tumors, on the other hand, it seems likely that variations in lipid content are linked with endocrine metabolism and hormone storage. It, then, is of a different nature from the degenerative lipids of dysgerminomas. In the tumor herein described there was not an unusual amount of endocrine activity, but much evidence grossly and microscopically of degenerative changes. In the light of the work just mentioned this would suggest that there should be little lipid storage of a hormonal nature, but there may well have been deposits of a degenerative nature, thus resembling the lipids of dysgerminomas. The explanation for pseudotubercles in our case may, therefore, be similar to that offered for the presence of these structures in dysgerminomas.

Föderl,<sup>20</sup> in cases of dysgerminomas, has found pseudotubercles in tumor thrombi lying within the lumina of small blood vessels containing no stromal elements. Under these circumstances a derivation from tumor cells or from transformed endothelial cells must be assumed.

#### SUMMARY

A case of granulosa cell tumor is reported which satisfied some of the criteria for malignancy in neoplasms of this group. It also presented the interesting and previously unemphasized histological finding of pseudotubercles within its structure. It is believed that the best explanation for the presence of pseudotubercles is that they represent a stromal reaction to lipids derived from disintegrating tumor cells.

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#### DESCRIPTION OF PLATES

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##### PLATE 112

FIG. 1. Gross picture of one ovarian tumor mass, measuring 18 by 15 by 8 cm. The outer surface was rough and slightly lobulated. The cut section presented a doughy structure with areas of necrosis, some hemorrhage, and a few cystic areas measuring from 1 mm. to 5.5 cm. in diameter.

FIG. 2. The neoplasm shows numerous Call-Exner bodies, some containing small ovum-like structures. The intervening structure is made up mainly of anaplastic granulosa cells.  $\times 200$ .

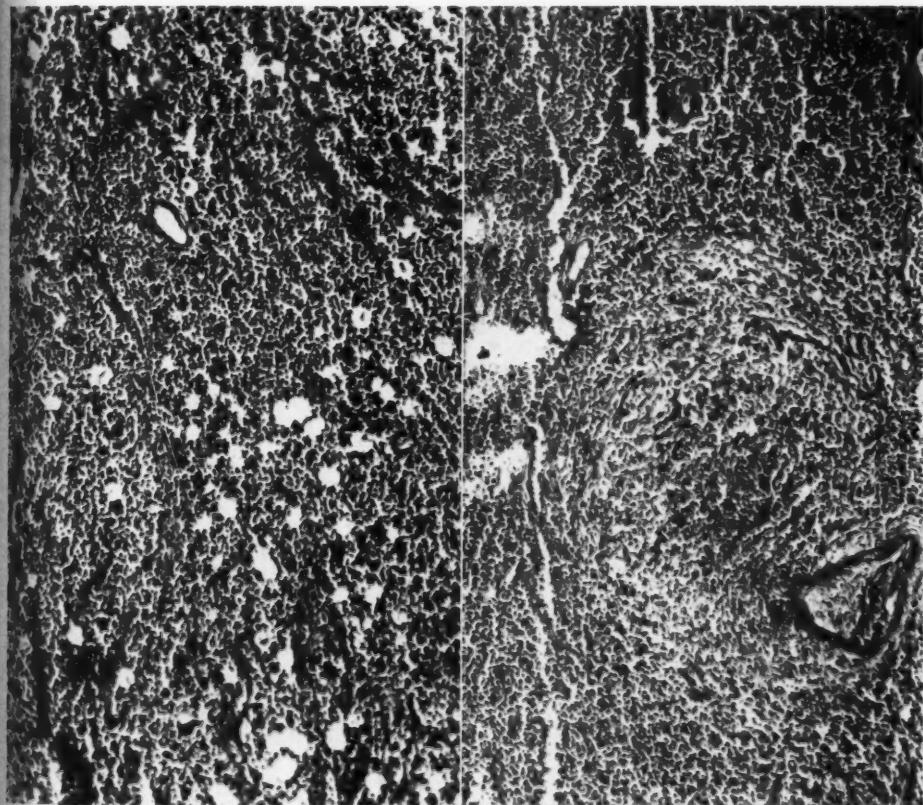
FIG. 3. A pseudotubercle with a small central area of necrosis. The tubercle is surrounded by an even distribution of granulosa cells.  $\times 200$ .







1



2

Schattenberg and Harris

3

Malignant Granulosa Cell Tumor

PLATE 113

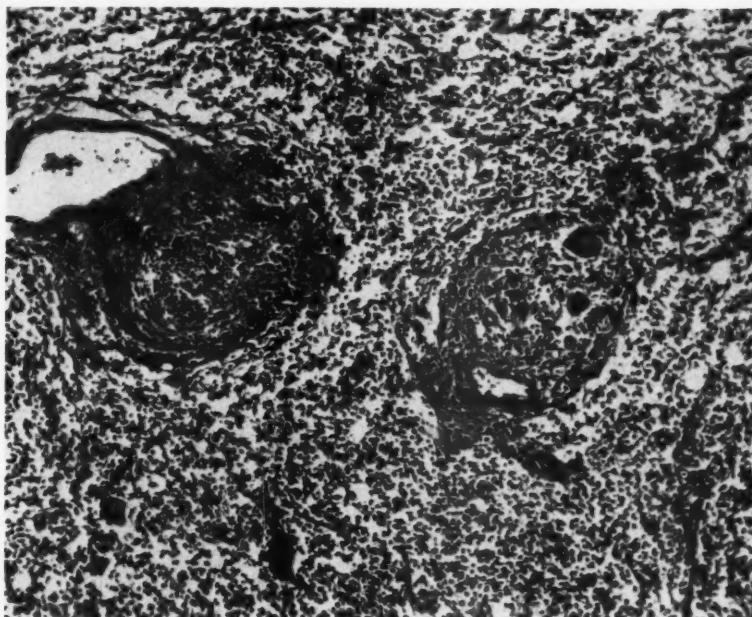
FIG. 4. Two pseudotubercles, one with two giant cells in its periphery. A few Call-Exner bodies are also shown in the surrounding masses of granulosa cells.  $\times 200$ .

FIG. 5. In the endometrium the glandular elements are in the proliferative phase. The endometrium is considerably thickened. A small amount of leukocytic infiltration, mainly lymphocytes, is noted.  $\times 200$ .

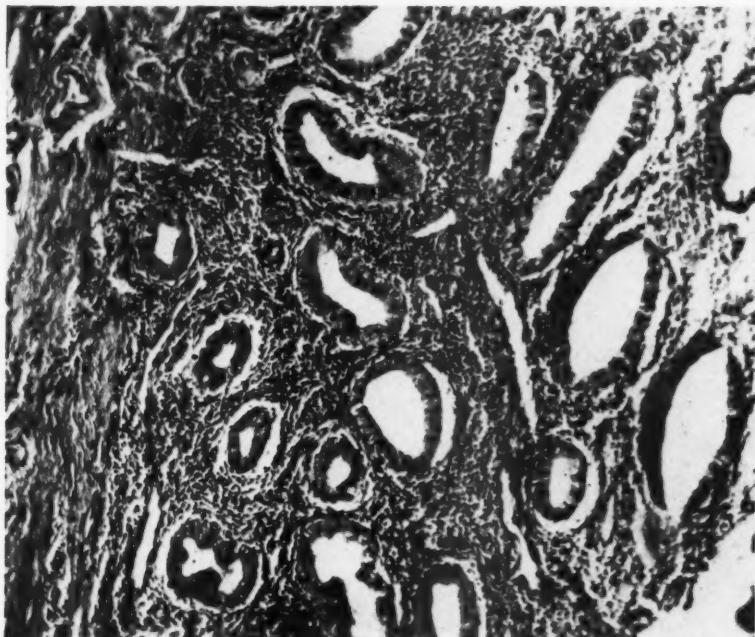




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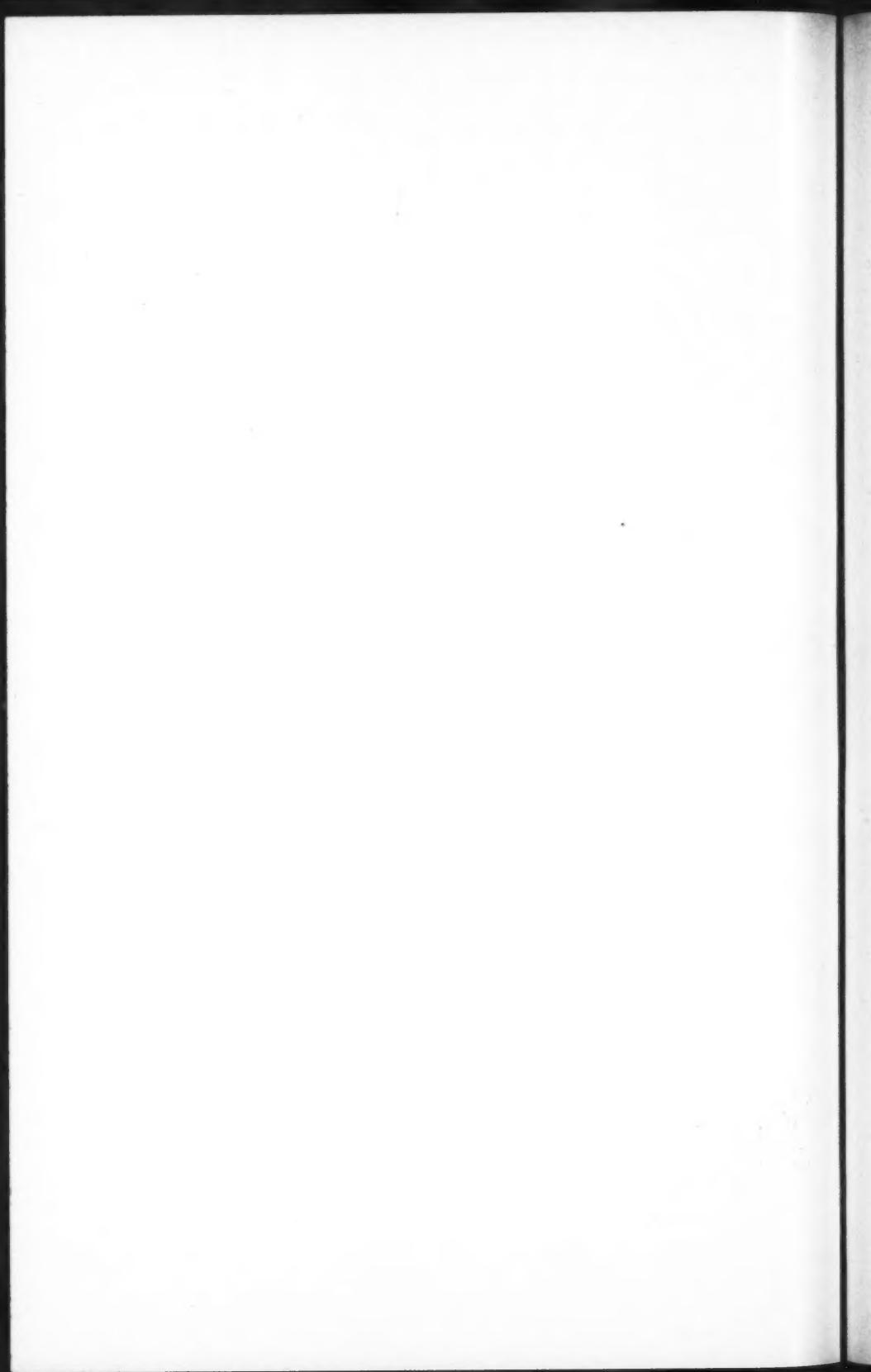


5



Schattenberg and Harris

Malignant Granulosa Cell Tumor



## DYSGERMINOMA OF THE OVARY\*

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The dysgerminoma<sup>†</sup> of the ovary is a solid tumor which was formerly thought to be quite rare, although more than 200 cases have been published. This tumor is of considerable interest because of the theories of its histogenesis, its potential malignancy, the enormous size it may attain in a child, and its frequent occurrence in pseudohermaphrodites. The name "disgerminoma," proposed by Meyer<sup>1</sup> in 1931, properly defines the tumor and eliminates confusion in terminology caused by describing it as embryonal carcinoma, seminoma, sarcoma, and under many other names. Barzilai's<sup>2</sup> definition is as follows: "The disgerminoma of the ovary is a germinal tissue tumor made up of cellular elements indistinguishable from the sexually undifferentiated mesenchymal cells of the early gonad."

The tumor is frequently found in childhood. Seegar,<sup>3</sup> in reporting 98 cases, found 44.5 per cent occurring in the ages between 10 and 19 years, with only 10 per cent in women over 40 years. The tumor is bilateral in approximately 28 to 30 per cent of cases, and tumor growth may progress in the remaining ovary subsequent to the surgical removal of one ovary, as occurred in one of my cases (case 1).

The dysgerminoma must be classified as a malignant tumor since it grows rapidly, spreads by implantation, and sets up distant metastases. Kirshbaum and Newman<sup>4</sup> reported the case of a 36-year-old female with metastases to the peritoneum, lungs, both kidneys, and the liver, and it has been estimated that about 25 per cent of cases have extrapelvic metastases at the time of operation.

Underdevelopment of the external or internal genital organs is sometimes associated with dysgerminoma. Novak and Gray<sup>5</sup> found only 3 of their 17 patients to have underdevelopment of the external or internal genital organs, although 4 others exhibited hirsutism, and one had a deep, masculine voice. Long, Ziskind, and Storck,<sup>6</sup> in describing a case of dysgerminoma occurring in a pseudohermaphrodite, pointed out that a large pelvic tumor in a patient with pseudohermaphroditism femininus and infantile genitalia suggests the presence of a dysgerminoma.

\* Received for publication, May 29, 1945.

† The spelling "dysgerminoma" is used in *The American Journal of Pathology*, but in direct quotations the original style will be preserved.

## GROSS DESCRIPTION

Dysgerminomata as found at operation or autopsy vary widely in size from small nodules to enormous tumors weighing more than 5 kg. (case 1). The smaller ones may retain the general shape of the ovary, the larger become spherical or ovoid. The tumor is usually encapsulated, with a smooth surface, although it frequently has a bosselated or knobby surface resembling brain in the gross appearance (Fig. 7). The surface made by cutting is yellow to gray, rather solid and homogenous, with areas of degeneration and hemorrhage in the larger tumors.

## MICROSCOPIC DESCRIPTION

The microscopic picture is usually characteristic: the cells are large and uniform in size, the cytoplasm is granular, the nucleus is large, round, and centrally placed. Basophilic nucleoli are frequently seen. Barzilai<sup>2</sup> stated that the true cellular detail is well seen only in frozen tissue or in celloidin preparations since shrinkage occurs when formalin fixation is used with embedding in paraffin. Thus the cells appear to be much smaller and to have indistinct borders, which has led to controversy regarding the type and has favored a diagnosis of round-cell sarcoma.

The pattern which the tumor cells assume varies from a cord-like arrangement, resembling abortive tubule formation, to a medullary or alveolar form, the tumor cells being separated into small or large clumps by fine connective tissue septa. Lymphocytes are frequently found in this connective tissue, and actual lymph follicles are occasionally seen. Giant cells, resembling the Langhans' cells of tuberculosis, are often found in dysgerminoma. These cells have concentrically-placed, multiple nuclei and are often seen in clumps of lymphocytes, together with epithelioid cells simulating tubercles. The pathogenesis of these giant cells has not been satisfactorily explained although it is generally agreed that there is no relation between them and tuberculosis.

The positive Friedman or Aschheim-Zondek test often found in patients with dysgerminoma is interesting since there is no clinical or laboratory evidence of male or female hormonal stimulation. Spielman and Morton<sup>7</sup> reported a hormonal bio-assay in a case of ovarian dysgerminoma and found a complete absence of estrogenic hormone although prolan A was present but unaccounted for. Some patients with a positive Friedman test have been mistakenly thought to be pregnant but roentgenographic studies usually correct the diagnosis. There is, however, no uniformity, and the comparative rarity of

dysgerminoma as compared with other ovarian tumors makes the test of doubtful diagnostic value.

#### REPORT OF CASES

##### *Case 1*

The patient, a white female, 14 years old (no. 11603),\* was first seen by her physician in October, 1939, complaining of enlargement of the abdomen. She had menstruated once at 12 years of age, and again at 13. She had otherwise been in a good state of health. Enlargement of the abdomen had been noted 6 weeks prior to examination, and about the same time her voice became of lower pitch and almost masculine. At that time she was given thyroid extract and theelin for amenorrhea.

Examination was negative except for the abdomen which was enlarged to a size suggesting an 8 months' pregnancy. The mass was in the midline but the borders were indefinite. There was no change in the breasts. The blood pressure was 110/60 mm. Hg; laboratory studies of blood and urine showed no abnormality except that the *Friedman test* was positive.

At operation a solid right ovarian tumor was removed which weighed more than 4 kg. The left ovary was normal in size and appearance.

The tumor was an ovoid mass, measuring 28 by 15 by 12 cm., weighing 4028 gm. The surface was lobulated, yellowish pink and in some areas dark red. There was a pedicle, approximately 8 cm. in length. The surface made by cutting showed multiple nodules made up of soft yellow to yellowish pink tissue, varying in size from 0.5 to 7 cm. in diameter. The tissue between these nodules was firm and fibrous (Fig. 1). The sections showed large cells, arranged in semi-cords, separated by fine connective tissue. Some of these were in a medullary pattern, with giant cells and lymphocytes in the connective tissue stroma (Figs. 2 and 3). The diagnosis was dysgerminoma.

Subsequent roentgenograms of the chest and pelvis showed no evidence of metastases. The patient made an uneventful recovery, and left the hospital on the tenth postoperative day.

The patient was seen at intervals, without complaint, until March, 1943, at which time she was 18 years old. She complained of pain in the right lower quadrant of the abdomen. Her menstrual periods had been normal until 3 or 4 months prior to that time when they occurred every 2 to 4 weeks. Her last menstrual period had commenced 10 days before this examination, and she was menstruating when seen.

Pelvic examination showed a large mass in the left side of the pelvis. The examination was otherwise negative. *The Friedman test was negative.*

A laparotomy was performed, and a solid tumor, arising in the left ovary, was removed. This mass had broken through its capsule and was attached to the parietal peritoneum just below the umbilicus. There was no demonstrable involvement of lymph nodes, and no evidence of distant metastasis.

This second tumor was a solid mass measuring 16 by 10 by 10 cm., and weighing 1779 gm. The surface was nodular, bosselated, yellow to gray, with some dark red nodules. The surface made by cutting

\* Patient of Dr. Jerome Jacobs, Seattle, Wash.

showed soft areas with fibrous septa dividing them. There was evidence of recent hemorrhages. The soft areas cut with a fish-flesh-like consistency (Fig. 4). The sections showed a neoplasm made up of large round and polyhedral cells. They had small nuclei and granular cytoplasm. There were fine and coarse bands of connective tissue dividing these cells into groups. Otherwise they were in a medullary pattern. The diagnosis was dysgerminoma (Fig. 5).

The patient made an uneventful recovery and left the hospital on the eleventh postoperative day. She subsequently received x-ray therapy totalling 8200 r. over four pelvic ports. She has been seen at intervals and there has been no evidence of recurrence or metastasis.

#### Case 2

The patient was a female, 11 years old (clinic no. 21203),\* who had been seen in a neighboring city on February 3, 1945, with the chief complaint of pain in her abdomen. The child had always been an active healthy schoolgirl whose family had noticed a gradual enlargement of her abdomen for several months. Two days prior to admission to the hospital the child had struck her abdomen against the corner of a desk, following which her abdomen became progressively larger and there was increasing pain. She had never menstruated.

On examination this child showed an enlargement of the abdomen resembling an 8 months' pregnancy. Percussion and palpation showed the enlargement to be due in part to free fluid. Because of the apparent emergency, a Friedman test was not done. Laparotomy was done promptly and a tumor was found occupying the pelvis and lower abdomen. There were 2 to 3 liters of clear straw-colored fluid in the peritoneal cavity, and a large tumor was found arising in the right ovarian region. This was easily removed. There was no evidence of extension or implantation. The left ovary and the remainder of the pelvic genitalia were entirely normal. Recovery was uneventful.

The specimen was a solid, rounded and flattened tumor, measuring 21 by 10 by 10 cm., and weighing 1500 gm. The capsule was smooth; the surface was bosselated and the consistency was soft. It was yellow to gray and red, and the surface made by cutting showed many soft areas which were yellow and cut with the consistency of fish flesh. There were several large pockets containing fluid and clotted blood where the tumor had undergone degeneration. The sections showed a neoplasm with relatively few tumor cells and a preponderance of fibrous connective tissue; the tumor cells were round or polyhedral, and were arranged in small medullary clumps; there were many lymphocytes in the connective tissue and some giant cells (Fig. 6). The diagnosis was dysgerminoma.

#### Case 3

The patient, a female, 18 years old (clinic no. 114698),† came to the Mason Clinic on January 30, 1945, complaining of soreness in the abdomen of 3 months' duration. The soreness had radiated to the inguinal region on both sides. She

\* Case of Dr. Lawrence Schuler, Port Angeles, Wash.

† Case of Dr. Joel W. Baker, Seattle, Wash.

consulted a physician who prescribed some medicine, but subsequently the patient noticed a slight abdominal swelling unaccompanied by pain.

On February 2, 1945, she fell and immediately afterwards had an acute episode of abdominal pain. She then consulted a doctor who felt that she was pregnant, although a Friedman test was negative. The patient's last menstrual period started on February 2, 1945. Examination showed a large tumor filling the lower abdomen and extending to the umbilicus. Rectal and vaginal examinations showed the cervix to be normal, and a diagnosis of ovarian tumor was made.

Laparotomy was performed on February 9, 1945, and a tumor was found arising in the left ovary and extending from the pelvis into the left flank. The left fallopian tube was long and edematous. The right ovary appeared entirely normal. The tumor was removed without difficulty, and the patient made an uneventful recovery.

The tumor was solid, measuring 21 by 13 by 10 cm., and weighing 1376 gm. The surface resembled the surface of a brain, with deep convolutions, although some of them were flattened. The surface made by cutting showed many nodules which were soft and cut with fish-flesh-like consistency. The tissue between these nodules was firm and fibrous (Fig. 7). Sections showed the tumor cells to be pleomorphic. They had a granular cytoplasm, and there were many mitotic figures. The tumor cells were separated by fine fibrous connective tissue. Many lymphocytes were present, some of them in large clumps resembling a lymph follicle (Fig. 8). The diagnosis was dysgerminoma.

#### SUMMARY

Four instances of dysgerminoma of the ovary are reported, arising in three females. The bilateral tumors of case 1 were not simultaneous. The left ovary appeared entirely normal at the time of the removal of an enormous tumor arising in the right ovary. The second tumor was removed nearly 4 years later.

A Friedman test was done in three cases; it was positive in 1, negative in 2.

In case 2 the tumor was accompanied by considerable ascites which was of recent origin since the abdomen enlarged rapidly following an accident. The presence of ascites has been commented on in previous reports.

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#### DESCRIPTION OF PLATES

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##### PLATE 114

FIG. 1. Case 1. A cut section of the dysgerminoma, weighing 4028 gm.

FIG. 2. Case 1. Photomicrograph showing a typical field, demonstrating a cord-like or semitubular arrangement. Hematoxylin and eosin stain.  $\times 115$ .





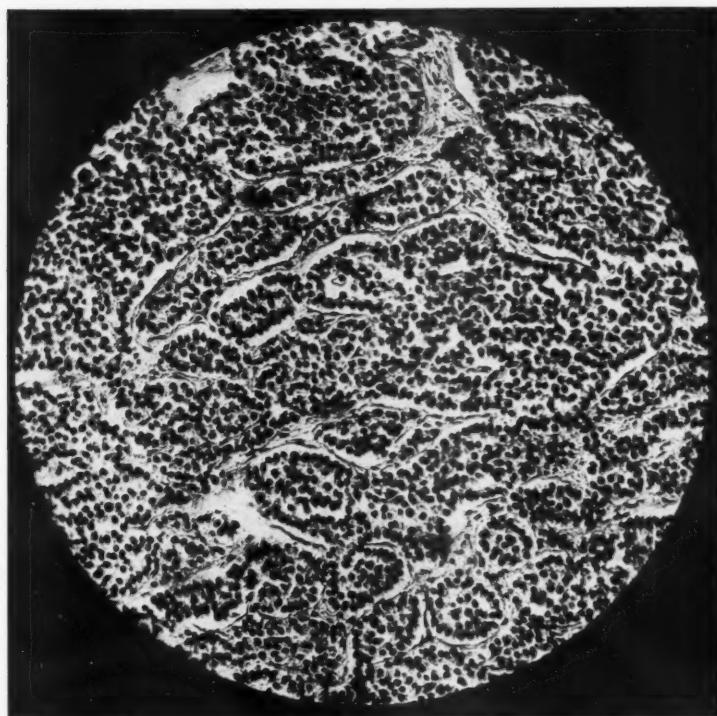


PLATE 115

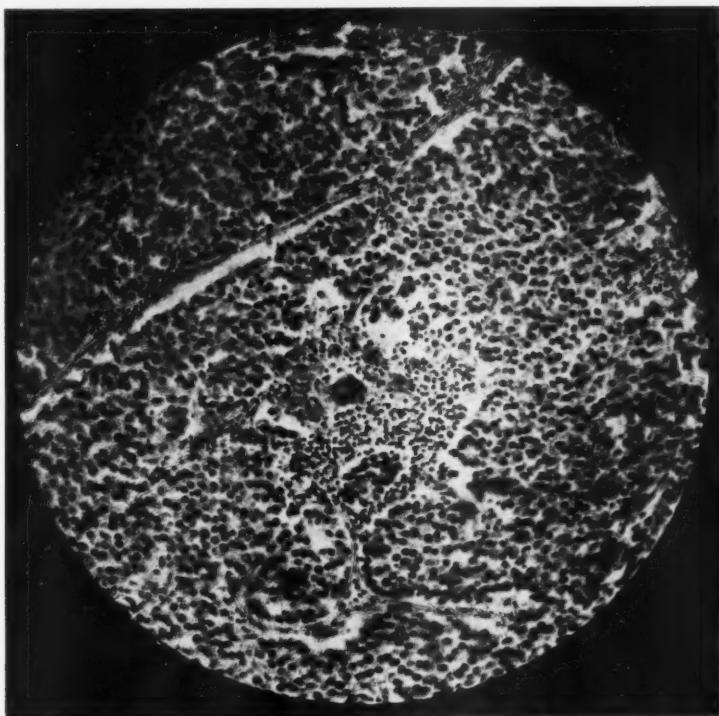
FIG. 3. Case 1. Photomicrograph showing a giant cell surrounded by lymphocytes. Hematoxylin and eosin stain.  $\times 116$ .

FIG. 4. The gross appearance of the second ovary in case 1.





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Potter

Dysgerminoma of the Ovary

PLATE 116

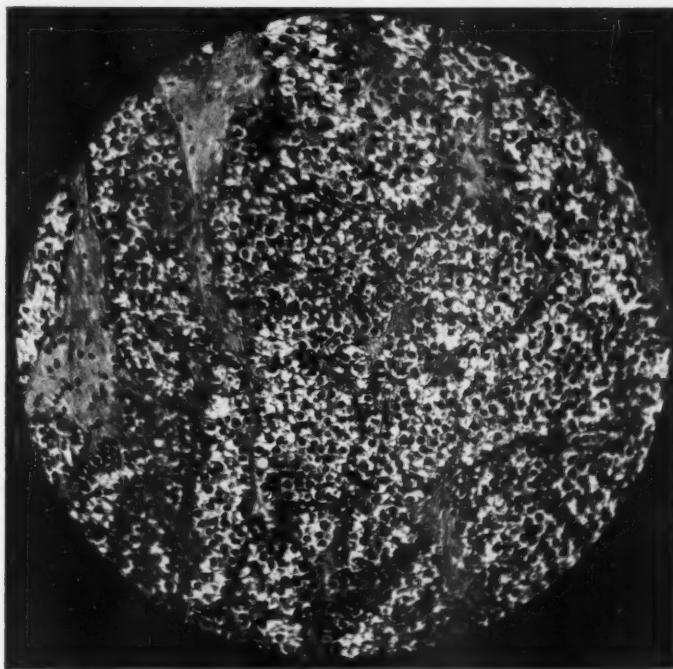
FIG. 5. Case 1. Photomicrograph of tumor shown in Figure 4, showing a more undifferentiated tumor than in Figure 2. Hematoxylin and eosin stain.  $\times 107$ .

FIG. 6. Case 2. Photomicrograph showing a predominance of fibrous connective tissue with many lymphocytes in the stroma. Hematoxylin and eosin stain.  $\times 107$ .

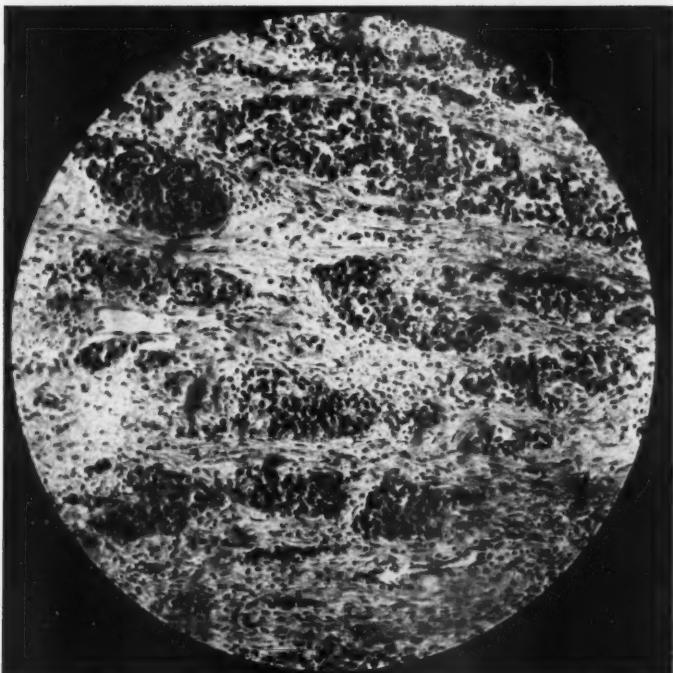




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Potter

Dysgerminoma of the Ovary

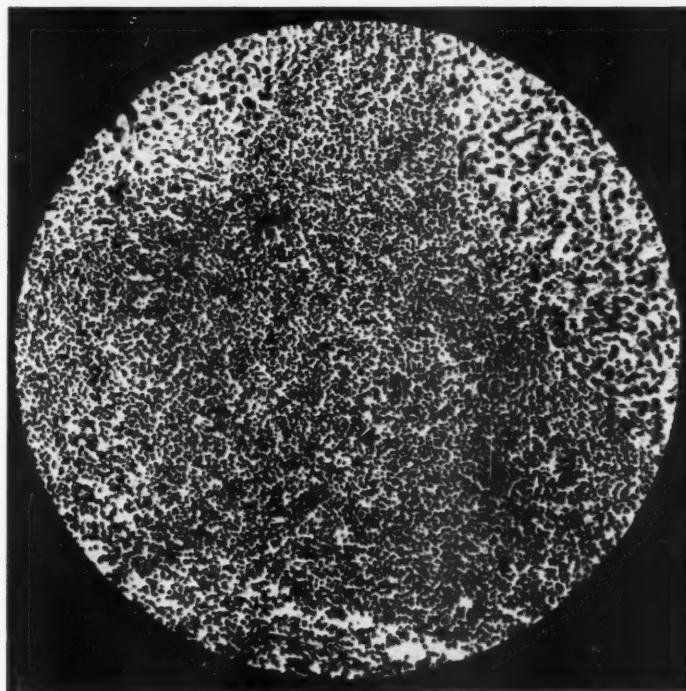
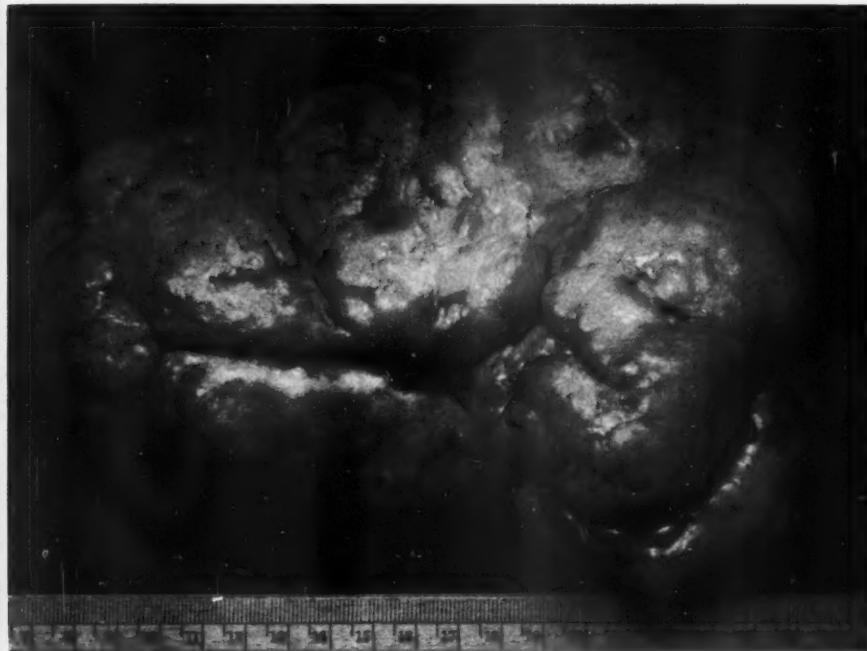
PLATE 117

FIG. 7. Case 3. Gross appearance of the tumor, showing a similarity to the surface of a brain.

FIG. 8. Case 3. Photomicrograph, centering over a clump of lymphocytes with tumor cells at the periphery. Hematoxylin and eosin stain.  $\times 110$ .







Potter

Dysgerminoma of the Ovary



## OBLITERATIVE CEREBRAL ARTERIOSCLEROSIS A CHARACTERISTIC VASCULAR SYNDROME \*

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Cerebral arteriosclerosis is the most common and one of the least understood pathologic conditions of the brain. There is no direct relationship between the severity of clinical symptomatology and the degree of sclerosis of arteries in the brain. There are many cases in which, in spite of a far advanced sclerosis of the larger arteries, clinical manifestations are insignificant. On the other hand, in cases with obvious clinical symptomatology considered to be characteristic of cerebral arteriosclerosis, the gross pathologic findings may be minimal or absent.

Possibly the reason for this apparent discrepancy of clinical and anatomical findings has been that most attention was devoted to alterations of the larger arteries; interest has been focused on the gross lesion of arteriosclerotic plaque formation. While this common form of arteriosclerosis has been relatively well studied, little attention has been devoted to the histologic changes of the smaller blood vessels in cases of cerebral arteriosclerosis. These lesions require further detailed study.

In an earlier investigation of vascular changes in 25 cases of hypertensive brain disease an attempt was made to describe a special form of arteriolar alteration typical of hypertension.<sup>1</sup> The view was expressed that these lesions of the arterioles should be separated from the total group of arteriosclerotic changes under the heading of "hypertensive hyaline arteriolopathy."

This presentation will describe a characteristic vascular change frequently observed in cases with clinical manifestations of cerebral arteriosclerosis. This abnormality may be found in combination with sclerosis of larger arteries, or independent of it.

### MATERIAL AND METHODS

This report is based on a detailed study of the cerebral vascular changes in 10 cases of arteriosclerosis. Sections from numerous areas of the brain were stained by the hematoxylin and eosin, cresyl violet, Loyez myelin sheath and Bodian protargol methods, and in some instances with scarlet red stain for fat.

The vascular lesions which are to be described as "obliterative

\* Received for publication, May 29, 1945.

cerebral arteriosclerosis" were encountered in older patients, the ages varying between 68 and 94 years. Both sexes were equally represented. Mental disturbances characterized as those of senile dementia were noted in 5 cases of this series (50 per cent of all cases). Signs of disseminated cerebral lesions (a multiplicity of neurologic signs) were present in 4 instances. In only one case was arterial hypertension (220/120 mm. Hg) noted. In 4 cases there were clinical symptoms of arteriolonephrosclerosis. Evidences of vascular lesions in the peripheral circulation also were found, *i.e.*, in 3 cases there was gangrene of a big toe.

#### PATHOLOGIC OBSERVATIONS

*Gross Findings.* The gross findings in the brain were quite uniform in character. The leptomeninges were often thickened, opaque, and slightly adherent to the underlying cortex. The superior surface of both hemispheres disclosed a moderate degree of convolutional irregularity caused by the presence of numerous minute foci of softening or glial scar formation. Areas of normal tissue frequently alternated with small areas of scarring or with small foci of cystic softening (Fig. 2), producing a slightly granulated appearance of the cortical surface (Fig. 1). The small glial scars often appeared on the surface as tiny depressions. The large vessels at the base were in some cases normal. In others they appeared enlarged, irregular, and tortuous; their walls presented extensive atheromatous change and contained numerous arteriosclerotic plaques. The smaller cortical vessels appeared slightly thickened and tortuous. The larger cisternal as well as the subarachnoid spaces appeared considerably widened.

Coronal sections of both hemispheres revealed in all instances strip-like areas of softening of the cortical ribbon, frequently associated with diffuse atrophy of the cortical gray matter. In some portions of the latter, small areas of softening were so numerous as to give the cortex a mottled appearance. The white matter was grossly normal except for occasional small areas of cystic softening or glial scars varying considerably in size, number, and location. The ventricles were enlarged in all instances.

*Microscopic Findings.* In all cases the leptomeninges were moderately thickened. The subarachnoidal space was distended and harbored small accumulations of fat granule cells. In 4 cases there was dense focal infiltration of the leptomeninges with gitter cells; adjacent portions of the meninges revealed no infiltration. The gitter cell accumulation in the leptomeninges was secondary, as a rule, to numerous small areas of softening localized in the upper layers of the cortical ribbon and communicating with the subarachnoidal space.

The cerebral cortex showed vascular lesions and changes in the parenchyma. The main histologic alterations of the vascular system are illustrated by Figures 3 to 7. The majority of the small arteries exhibited a conspicuous thickening of the intima. The latter showed two principal types of alteration: proliferative and necrobiotic. In the early proliferative stage, as illustrated in Figure 3, the changes were characterized by a cellular proliferation chiefly confined to the inner layer of the vessel. The subendothelial portion of the intima was thickened and very cellular, mainly because of the presence of granulation tissue rich in fibroblasts and mononuclear elements. The tremendous expansion and hyperplasia of the ground substance of the subendothelial connective tissue led to considerable narrowing of the vessel lumen. It was by no means rare that the lumen of the blood vessels was reduced to a mere slit. Cross sections of some of the blood vessels revealed the misshapen and considerably reduced vessel lumen bounded on the outer side by a relatively well preserved media and adventitia (Fig. 4). The proliferative changes, as a rule, were confined to the inner coat, bulging the intima outward and encroaching greatly upon the lumen. The lining endothelial cells showed mild proliferative and no degenerative changes. In addition there was a considerable increase and splitting of the inner elastic membrane in the form of concentrically arranged elastic laminae. Degenerative changes such as hyalinization of the intima or atheromatous plaque formation were not observed.

In more advanced stages (Figs. 5 to 7) the cells of the subendothelial part of the intima had undergone complete degeneration and necrosis and were replaced by a thin network of connective fibrils harboring a very few fibroblasts and macrophages. These alterations were occasionally associated with secondary atrophy of the media. They were very rarely complicated by fatty or mucoid degeneration. Sclerotic plaque formation or calcification was not observed. As a rule the adventitia was of normal appearance.

Changes of the nervous tissue secondary to the vascular alterations consisted of circumscribed focal areas of softening characterized by the presence of large numbers of fat granule cells (Fig. 2); in addition there were numerous areas of glial scar formation. These focal lesions resulting from insufficient blood supply were disseminated throughout the cortical ribbon (Fig. 1). Only occasionally were they found in the white matter and basal ganglia. In the upper layers of the cortex there were numerous stripe-like devastated areas in which all nerve cells had been destroyed and replaced by glial scar formation. All changes of the nervous tissue were associated with extreme narrowing

or complete occlusion of the vessel lumina. Their variance in severity might be explained by variation in rapidity and completeness of the circulatory disturbance, and by the original caliber of the damaged blood vessel.

The examination of sections taken from other viscera (kidney, spleen, pancreas, liver, lungs, and heart) revealed a moderate degree of arteriosclerosis. Vascular alterations similar to those described as "obliterative cerebral arteriosclerosis" were not observed.

#### COMMENT

Most investigators seem to focus their attention on two types of arteriosclerotic alteration. The first is atherosclerosis or atheroma, which usually affects the larger cerebral arteries and is characterized by patchy degeneration of the intima with local reaction of the media. There occurs a deposition of calcium in the involved portion of the vessel wall. Yellow nodules are found both on the outer and inner surfaces of the arteries in the circle of Willis; these may cause considerable narrowing or complete occlusion of the vessel lumen. Occasionally fatty degeneration of the intima may cause rupture of the inner coat subsequent to which an atheromatous "ulcer" is formed. The damage to the lining endothelium may lead to thrombus formation.

The second type of arteriosclerotic vessel change is less well defined and involves the medium-sized arteries and arterioles and is described as "hyperplastic sclerosis" or "arteriolosclerosis." Histologically, this process is characterized by diffuse thickening of the *entire* vessel wall with a corresponding decrease in the size of the lumen. There is usually considerable hypertrophy of the media and hyperplastic thickening of the intima, and the latter is usually associated with a reduplication of the elastic membrane. In addition there is considerable proliferation of fibrous tissue which gradually replaces the media and adventitia. Degenerative changes of the intima such as hyalinization or fatty degeneration are quite common.

In spite of the vague morphologic resemblance between the so-called "hyperplastic sclerosis" (the second type of arteriosclerosis) and the alterations described in this study, there are significant differences between the two conditions. These might be summarized as follows: (1) The vascular alterations in hyperplastic sclerosis involve the *entire* vessel wall, including media and adventitia. In the lesions described in the present study the essential changes are confined to the inner layer of the vessel wall. The media and the adventitia did not reveal any marked pathologic alteration. (2) Although intimal pro-

liferation occurs in both conditions, in "hyperplastic sclerosis" this proliferation is usually associated with degenerative changes such as hyalinization or fatty degeneration, changes which were not observed in the cases included in this study. (3) Marked cellular proliferation of the subendothelial connective tissue, a frequent observation in the early stage in my cases, has not been described in hyperplastic sclerosis.

It seems proper to conclude that the vascular lesions described in this study represent a characteristic type of sclerosis of the small cerebral arteries, which can be differentiated without difficulty from hyperplastic sclerosis or from the so-called arteriosclerosis. It is proposed to designate this vascular alteration as "obliterative arteriosclerosis." This term indicates that the main pathologic process consists of a tremendous expansion of the intima, the effect of which is an almost complete obliteration of the vessel lumen.

Confusion with thrombo-angiitis obliterans (Buerger's disease<sup>2</sup>) does not seem likely. In spite of the apparent similarity of the two conditions, the following differential features should be noted: (1) In thrombo-angiitis obliterans the proliferative process of the intima is usually associated with thrombus formation; this is not a common occurrence in "obliterative arteriosclerosis." (2) In thrombo-angiitis obliterans the proliferative changes of the intima are seldom complicated by typical arteriosclerotic changes such as splitting or reduplication of the elastic membrane, findings frequently present in "obliterative arteriosclerosis." (3) The intramural hemorrhages frequently observed in the early stage of thrombo-angiitis obliterans<sup>3</sup> have not been seen in the vascular alterations described in this study.

It is generally known that a certain degree of reactive intimal proliferation may take place in periarteritis nodosa.<sup>4</sup> Confusion with "obliterative arteriosclerosis" does not seem likely; severe inflammatory changes of the entire vascular wall associated with necrosis of the subendothelial connective tissue and adjacent media, characteristic of periarteritis nodosa, were not observed in lesions described in the present study.

Von Glahn and Pappenheimer<sup>5</sup> described specific lesions of peripheral blood vessels in 10 cases of rheumatic cardiac disease. Although in the chronic stage the vascular alterations may be similar to those of obliterating endarteritis, there are significant differences between the two conditions. The vascular lesions described by Von Glahn and Pappenheimer are characterized by exudation of fibrin into and about the blood vessel, by destructive changes in the cellular components of the entire vessel wall, and finally by a distinctive cellular

reaction in the adjacent tissue. None of these alterations were observed in the vascular changes described in the present study.

Lesions similar to those described by Von Glahn and Pappenheimer<sup>5</sup> were reported by Karsner and Bayless<sup>6</sup> in the internal organs, and by Gross, Kugel, and Epstein<sup>7</sup> for the myocardial arteries. Similar vascular alterations of the cerebral blood vessels were described by Bruetsch.<sup>8</sup> The vascular changes were chiefly confined to the meningeal and cortical vessels and were characterized by proliferation of endothelial cells, leading to partial or complete occlusion of the vessel lumen. In addition fibrin plugs were observed into which endothelial cells were growing. In each instance these changes bear very little resemblance to "obliterative arteriosclerosis." The absence of endothelial proliferation and fibrin plugs in the cases described in this study is sufficient to exclude vascular lesions of this category.

Obliterative arteritis is frequently seen with syphilis of the brain. It is well known that in cases of syphilis the intima may have proliferated and become thickened, giving the appearance of endarteritis. However, the differentiation from "obliterative arteriosclerosis" is not difficult. In syphilitic arteritis all three layers of the blood vessel are affected; the adventitial tunic and its spaces are infiltrated with lymphocytes and plasma cells, the adventitial cells themselves having proliferated. None of these changes are present in "obliterative arteriosclerosis."

#### SUMMARY

Distinctive alterations of the smaller blood vessels as found in the brains of 10 cases with cerebral arteriosclerosis are described. This lesion is characterized by tremendous expansion of the intima, resulting in narrowing or complete obliteration of the vessel lumen. It is proposed that this process be designated as "obliterative arteriosclerosis," and considered as a special type of arteriosclerosis of small cerebral blood vessels. Emphasis is placed on differentiation from "hyperplastic sclerosis."

Histologic changes in the parenchyma of the brain, particularly the cortical gray matter, consisted of diffusely scattered, stripe-like, small, old and recent softenings secondary to the obliterative vascular lesions.

A gross finding in the brain which was regarded as characteristic of "obliterative arteriosclerosis" was a granulated appearance of the cortical surface, due to numerous focal areas of glial scarring, often associated with stripe-like areas of softening involving the upper layers of the cortical ribbon.

"Obliterative arteriosclerosis" may occur independently of arteriosclerotic changes of the major cerebral arteries.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 118

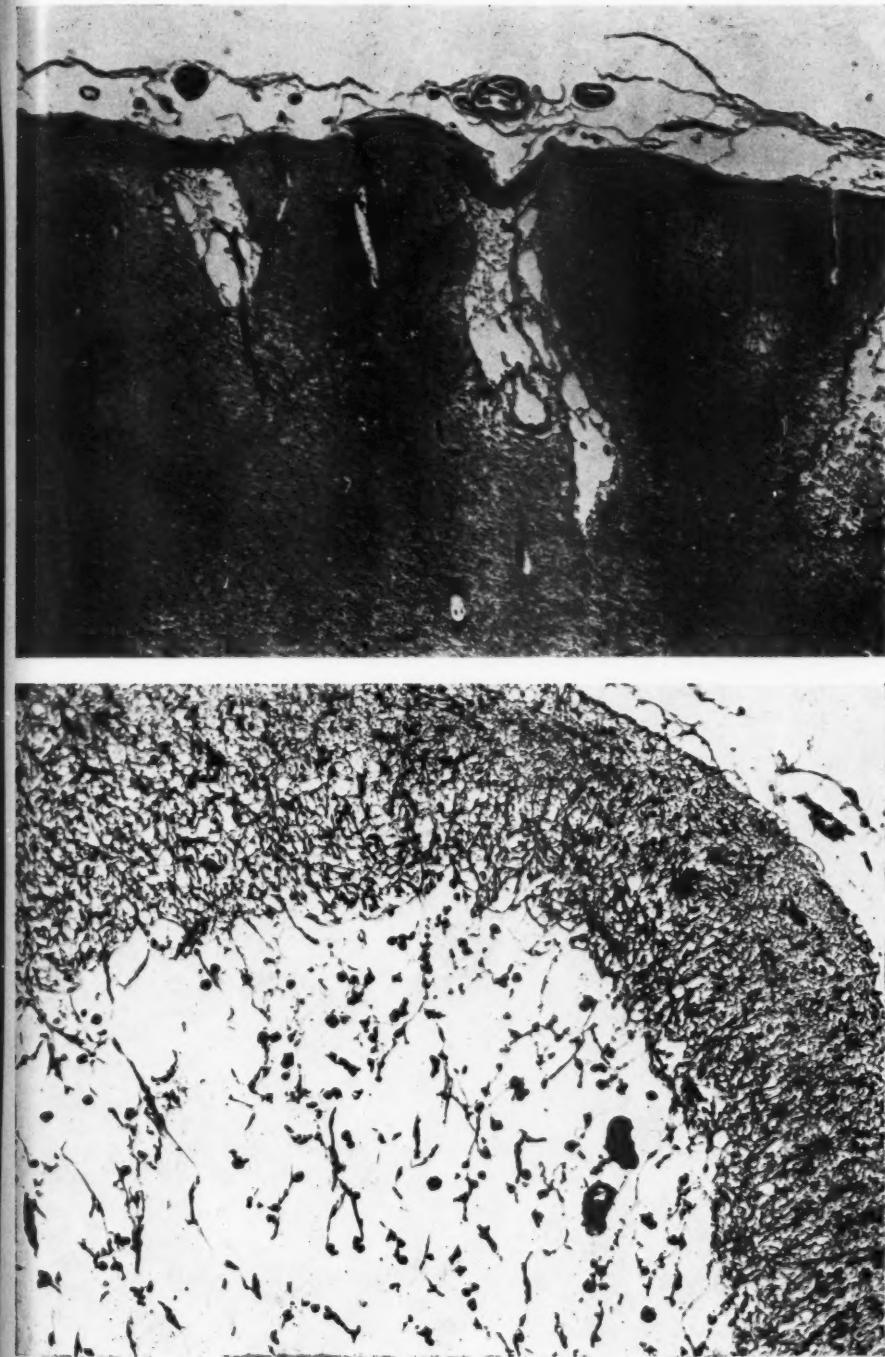
FIG. 1. Numerous small foci of cystic softening and glial scar formation diffusely scattered throughout the upper layers of the cortical ribbon, producing a granulated appearance of the cortical surface. Hematoxylin and eosin stain.  $\times 60$ .

FIG. 2. Small area of cystic softening of the cortical gray matter, containing numerous gitter cells. Cresyl violet stain.  $\times 115$ .

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Scheinker

Obliterative Cerebral Arteriosclerosis

PLATE 119

FIG. 3. Early proliferative stage of the characteristic lesion, confined chiefly to the inner layer of a small artery. Hematoxylin and eosin stain.  $\times 115$ .

FIG. 4. Small meningeal artery with a considerably reduced lumen resulting from an expansion of the ground substance of the subendothelial connective tissue. The media and adventitia are relatively well preserved. Hematoxylin and eosin stain.  $\times 130$ .

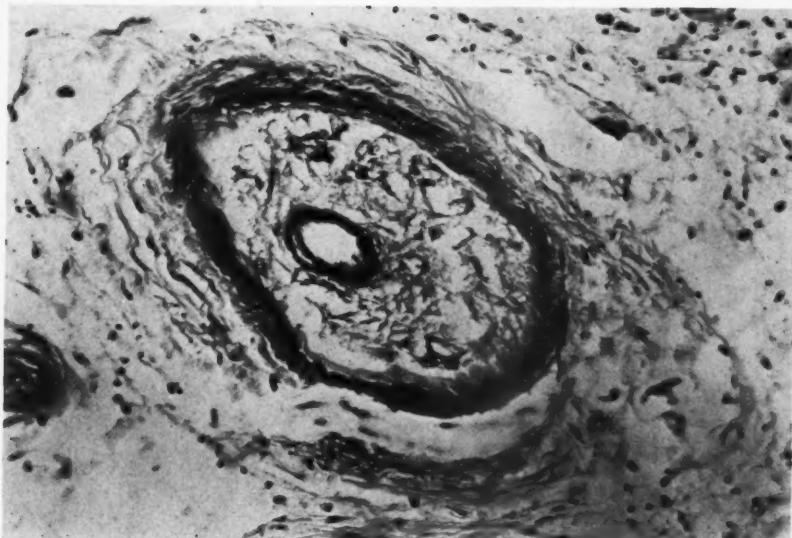
FIG. 5. Advanced necrobiotic stage of the lesion with tremendous expansion of the intima, and consecutive, almost complete, obliteration of the vessel lumen. Hematoxylin and eosin stain.  $\times 130$ .







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Scheinker

Obliterative Cerebral Arteriosclerosis

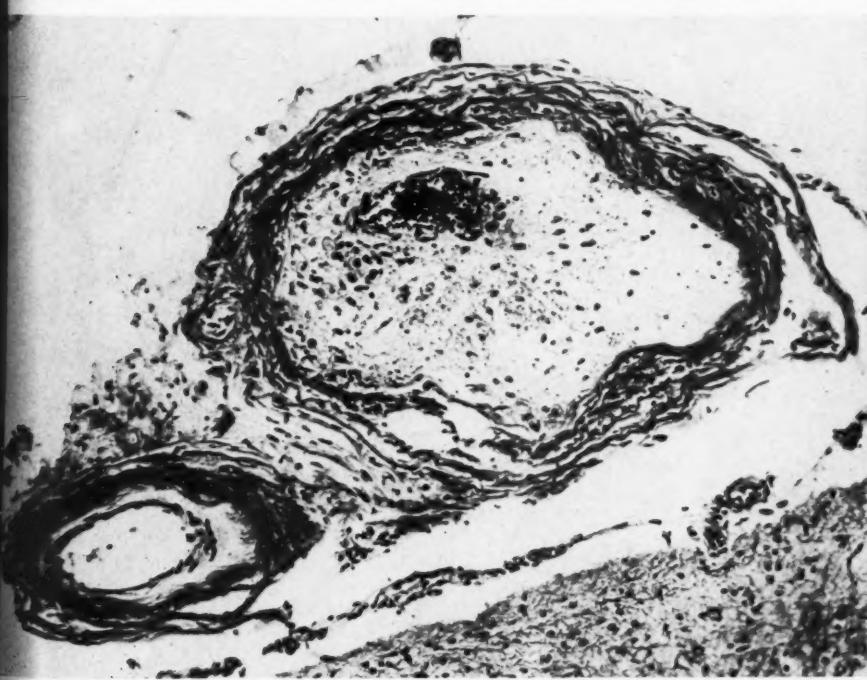
PLATE 120

FIG. 6. Two small arteries with tremendous thickening of the intima. The lumina are reduced to mere slits. Hematoxylin and eosin stain.  $\times 165$ .

FIG. 7. A small meningeal artery with a tremendous expansion of the subendothelial connective tissue and almost complete obliteration of the lumen. Hematoxylin and eosin stain.  $\times 165$ .

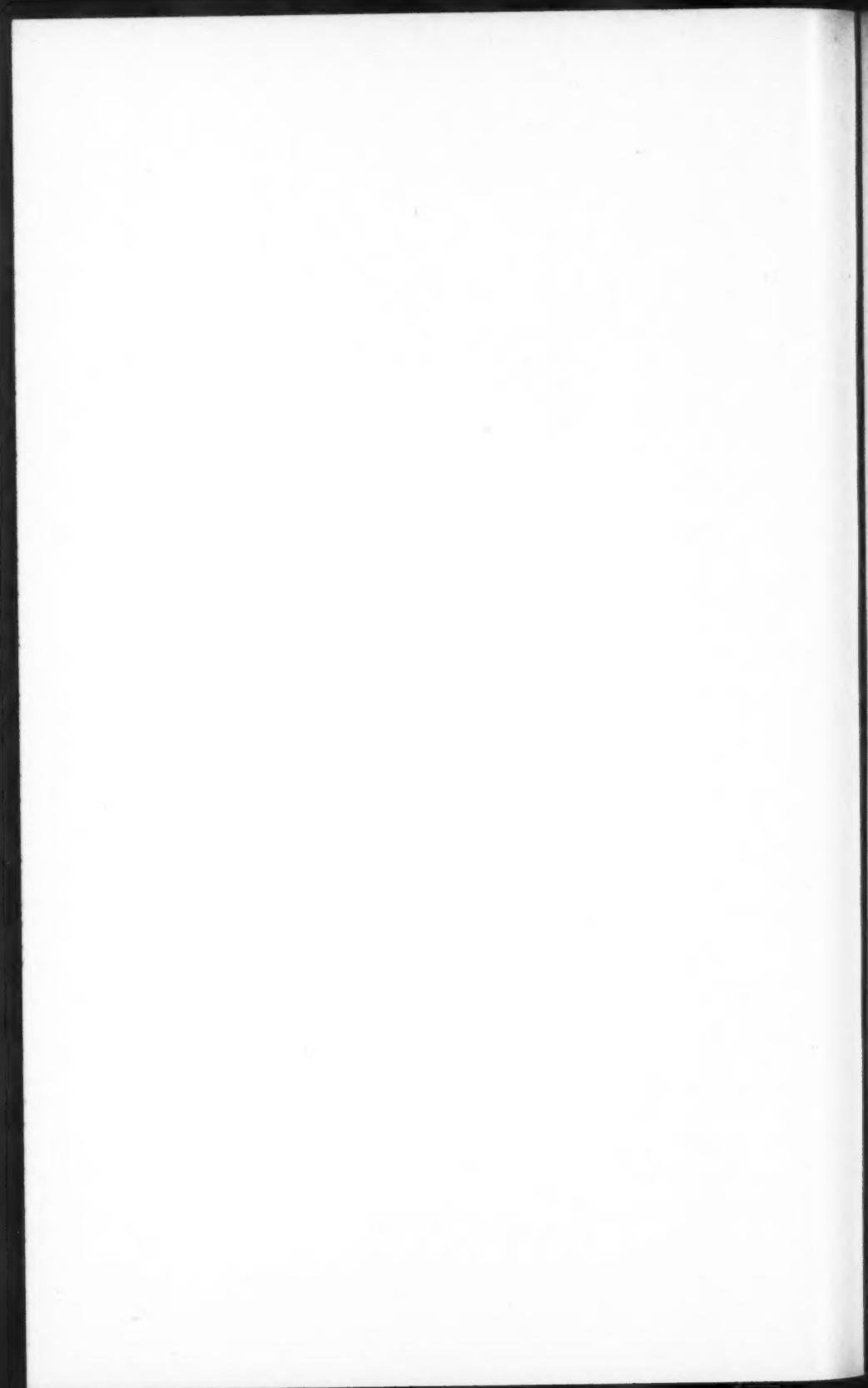






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Obliterative Cerebral Arteriosclerosis



## THE CENTRAL NERVOUS SYSTEM IN PNEUMONIA (NONSUPPURATIVE PNEUMONIC ENCEPHALITIS)

### II. A PATHOLOGIC STUDY \*

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(From the Department of Neuropsychiatry, University of Minnesota Medical School, Minneapolis, Minn.)

Although an abundant literature has accumulated on the subject of pneumonia, almost none of the published articles have considered the possible complications of the nervous system in this disease. This omission suggests the indifference with which this extremely important neurologic condition has been handled and indicates that perhaps many cerebral manifestations in pneumonia are being overlooked. In spite of the numerous organisms that are capable of producing a pneumonia, the clinical picture of the cerebral complications appears to be fairly constant (Baker and Noran<sup>1</sup>). During the acute stage of the pneumonitis there may occur generalized complaints such as headache, vomiting, diplopia, and lethargy (Eschbach,<sup>2</sup> Reimann,<sup>3</sup> Bonaba, Marcos, and Mendivil de Agorio,<sup>4</sup> Gareiso and Sagreras<sup>5</sup>). Occasionally, the involvement of the nervous system is much more dramatic and is manifested by coma, convulsions, or severe delirium often associated with visual hallucinations (Eschbach,<sup>2</sup> Comby,<sup>6</sup> Stephan,<sup>7</sup> Navarro,<sup>8</sup> Piaggio Garzón,<sup>9</sup> Bernheim and Bonnefoy,<sup>10</sup> de Filippi and Fernández,<sup>11</sup> Nové-Josserand, Rougier, and Feuillade,<sup>12</sup> Prandi<sup>13</sup>). Most of these symptoms have been assumed to be secondary to pyrexia even though they may occur in mild cases in which the temperature has not been significantly high. Sporadic cases have been reported in the foreign literature in which varying neurologic complications have appeared during convalescence while the temperature was normal and the patient was assumed to be well on the road to recovery (Gareiso and Sagreras,<sup>5</sup> Comby,<sup>6</sup> Piaggio Garzón,<sup>9</sup> Nové-Josserand *et al.*,<sup>12</sup> Lesieur, Froment, and Garin,<sup>14</sup> Regine,<sup>15</sup> Barni,<sup>16</sup> Grenet, Isaac-Georges, and Desmarquest,<sup>17</sup> Mouriquand, Bernheim, and Boucomont<sup>18</sup>). In many of these patients the cerebral involvement left permanent residua in the form of motor weakness, ataxia, aphasia, and athetosis, or were severe enough to result in cerebral death (Reimann,<sup>3</sup> Stephan,<sup>7</sup> Piaggio Garzón,<sup>9</sup> Mouriquand *et al.*<sup>18</sup>).

While clinical reports are relatively few, detailed pathologic investigations of this condition are even more conspicuous by their absence. A few scattered case reports are available and in these the

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cerebral changes are mentioned only briefly. Guillain and Vincent,<sup>19</sup> and Fraenkel<sup>20</sup> emphasized the presence of pneumococci around scattered vessels. Fraenkel noted that tiny cerebral vessels had undergone endothelial proliferation resulting in vascular occlusion. Perivascular erythrocytes and leukocytes were also observed. Mollard and Du-four<sup>21</sup> mentioned petechiae in one of the their fatal cases. Adler,<sup>22</sup> in a study of 5 fatal cases of pneumonic encephalitis, reported changes varying from mild congestion to extensive perivascular infiltrations of leukocytes, hemorrhages, and even demyelinization. The cases with the more severe tissue changes were called "proliferative pneumonia encephalitis" while those with mere congestion or hemorrhage were designated as "toxic-pneumonia encephalitis." Perrone and Wright<sup>23</sup> observed small hemorrhages, perivascular cuffs of mononuclear cells, and an astrocytic proliferation in the brain of a patient who had virus pneumonia. Golden<sup>24</sup> and Ingleby<sup>25</sup> described similar lesions in cases of encephalitis secondary to atypical pneumonia, presumably due to a virus. Golden favored the term, hemorrhagic encephalopathy. Ingleby appears to have been the only investigator to report inclusion bodies in the brain.

The more complete pathologic studies have been those by Lépine,<sup>26</sup> Bonaba and Barberousse,<sup>27</sup> and Marinesco, Jonesco-Sisesti, and Stroesco.<sup>28</sup> In a 53-year-old male who developed hemiplegia following pneumonia, Lépine observed a single area of softening involving the left middle frontal gyrus. No microscopic studies were performed. Bonaba and Barberousse described in detail the cerebral alterations in a 12-year-old child who manifested convulsions during the acute phase of a pneumonia. At autopsy the most prominent changes were found within the basal nuclei, where there occurred marked ependymal proliferation and diffuse glial increase. There were some perivascular infiltrates forming incomplete collars around the vessels. Vascular congestion and hemorrhage were most prominent within the putamen. Scattered nerve cells within the basal nuclei showed acute changes consisting of chromatolysis and swelling. The cerebral cortex and white matter were surprisingly free of lesions. Marinesco and his co-workers reported very complete histologic studies in 3 cases of pneumococcus encephalitis. The lesions consisted of scattered plaques of demyelination which predominated in the subcortical and periventricular white matter. These plaques were often perivascular with the zone of tissue directly adjacent to the vessel being best preserved, giving the area of demyelination a ring-like arrangement. The neuroglia within these demyelinated areas was destroyed while

at the periphery a mild gliosis had occurred. There was a mild diffuse astrocytic proliferation throughout the white matter. Vascular changes were also very prominent, particularly in the involved areas. Many of the smaller vessels contained fibrin thrombi with intermixed red and white cells. Numerous vessels were surrounded by accumulations of red or white cells. The spinal cord showed a marginal demyelination but its nerve cells appeared intact.

Even though the sporadic reports recorded in the literature leave little doubt concerning the existence of a pneumonic encephalitis, the paucity of complete pathologic studies has left numerous gaps in our knowledge of the morphologic nature of the lesions. It was felt, therefore, that a careful study of the histopathologic findings in this condition was definitely indicated.

We have been able to collect the brains from 10 cases of pneumonic encephalitis in which complete autopsies were performed. In all instances there was unquestionable evidence of encephalitis established by histologic study. Since we are primarily interested in the pathologic alterations within the nervous system, an extensive consideration of the clinical manifestations does not seem indicated. However, the salient clinical features, the anatomic variety of pneumonia, and the nature of the etiologic agent are presented in table form (Table I).

Most of our cases occurred in infants, but no age group was excluded. Three patients were past middle age, being 45, 50, and 50 years of age. There was no preference for either sex. In infancy the symptoms resulting from the pneumonia were often mild and the cerebral manifestations tended to be rather late in developing, at times appearing after recovery from pneumonitis had begun. Such a sequence was well demonstrated in case 3. In half of the cases the clinical evidence of involvement of the nervous system seemed mild. In one case (case 2) encephalitis was completely overlooked clinically even though the microscopic examination of the brain left no doubt about the existence of definite cerebral involvement.

The lungs were examined carefully in all cases, but a detailed account of the microscopic findings is not pertinent to this study. From the anatomic standpoint there were 5 cases of broncho-, lobular, or interstitial pneumonia, 3 cases of a typical lobar involvement, and 2 examples of diffuse hemorrhagic pulmonary lesions showing associated edema and consolidation.

The etiologic agent varied. Four cases were characteristic of the recently popularized, atypical pneumonia, which is presumably of viral origin. In 4 others definite bacterial agents were found: nonhemolytic

TABLE I  
Summary of 10 Cases with Encephalitis Associated with Pneumonia

Case	Age and sex	Clinical features	Encephalitic manifestations	Anatomic type of pneumonia	Etiologic agent of pneumonia
1. H.F.	Male 6 months	Cough, low-grade fever, transient mildempyema and otitis; sulfathiazole without response for a brief period only	Irritability and restlessness	Bronchopneumonia (largely resolved); healed pleurisy; thrombosis of the small pulmonary arteries	Staphylococcus, coagulase positive
2. Baby L	Female Newborn	Irregular respiratory distress and fever; gradual failure and death after 1 month	None noted on chart	Hemorrhagic consolidation of both lungs	Lipoid pneumonia (estab- lished by sudan III staining)
3. Baby H.	Male 26 days	Cough, fever, pneumonia, respiratory difficulty	Convulsive phenomena, stupor, and death	Bronchopneumonia (largely resolved)	Virus (?)
4. E.C.	Male 50 years	Acute febrile state and pneumonia	Choked disks	Extensive lobar pneumonia	Streptococcus, nonhemolytic
5. F.P.	Male 50 years	Clinical lobar pneumonia; fulminating course	Delirium; nuchal rigidity	Bilateral hemorrhagic pneumonia	Streptococcus, nonhemolytic
6. A.F.	Female 1½ mo.	Cough, slight intermittent fever, thrush of nose and throat; death in respiratory failure	Moderate lethargy	Bilateral interstitial pneumonia; no thrush involving bronchial tree	Virus (?) (thrush of nose and throat)
7. A.F.	Male 4½ mo.	Mild pharyngitis, cough		Pneumonia, lobular	Not determined
8. Q.	Male 2 months	Cough, cyanosis, and fever		Pneumonia, lobular	
9. P.	Female 10 days	Rhinitis, mild irregular fever, cough, dysphagia and cyanosis	Lethargy	Bronchopneumonia	
10. E.A.	Female 45 years	Fever, cough, and pulmonary findings		Lobar pneumonia	Pneumococcus, (type VIII)

streptococcus (2), staphylococcus, and type VIII pneumococcus. Probably the most remarkable case of encephalitis was that following a typical lipoid pneumonia confirmed by Sudan III preparations.

In spite of the great variation in the severity and in the cause of the pneumonia, we found that the pathologic picture of the encephalitis was similar in all. The histologic findings in the various cases differed only in degree. For this reason it is possible to combine the observations from the entire group into one composite pathologic description. In all cases samples of tissue were taken from a number of scattered areas of the brain for microscopic examination. Each block was prepared by means of the following technics: hematoxylin and phloxine stain, Nissl (cresyl violet) stain, Weil stain for myelin, phosphotungstic acid hematoxylin stain for glial fibers and fibrin, Bodian stain for neurofibrils and axis cylinders, and Weigert-van Gieson method for elastic fibers, connective tissue, and smooth muscle.

#### PATHOLOGIC ALTERATIONS

On gross examination the external surface of the brain invariably showed a marked vascular congestion and usually an associated, patchy, brownish discoloration resulting from irregular, small, subarachnoid hemorrhages. In certain instances rather large areas of the cerebral hemispheres were obscured by subarachnoid hemorrhage. Coronal sections of the brain consistently demonstrated prominent congestion. In the more severe encephalitic involvement, large numbers of petechiae were disseminated throughout the entire cerebrum with a predilection for the subcortical white substance. In an occasional case, these petechiae occurred only in more or less localized portions of the brain and were most prominent in the cortical gray matter.

#### MICROSCOPIC OBSERVATIONS

Microscopic study of the nervous system in pneumonic encephalitis revealed a uniform pathologic picture except for differences in severity. Such variations were readily correlated with the clinical severity and duration of the encephalitic complication. In order to arrive at a better understanding of the morphologic features, the anatomic changes observed in the various stages of this illness will be described under the following headings: those of mild, moderately severe, and most severe pneumonic encephalitis.

##### *Mild Encephalitis*

Even the early and milder cases revealed very extensive thrombosis of the cerebral vessels. This finding constituted the most significant

pathologic feature, and often was the only microscopic manifestation. Vessels without thrombi were distended with blood. (Because perivascular hemorrhages and secondary parenchymal changes are generally absent at this stage, this thrombotic process frequently is overlooked and is regarded merely as post-mortem clotting.)

The small vessels, arterioles, venules, and capillaries, showed by far the most frequent thrombotic involvement. The process of vascular thrombosis could be traced readily through the various stages of its development. The earliest evidence of thrombus formation was the observation of platelets, fibrin, or erythrocytes adhering to the intimal lining of a cerebral vessel. Later, the marginal zone of the vessel was obliterated, leaving only a small central opening through which red blood cells continued to circulate (Fig. 1). This remaining central channel of the blood stream was steadily narrowed by the peripheral clotting until the vessel was completely obstructed.

Either before or shortly after the clotting completely obstructed the lumen, the erythrocytes within the thrombus quickly lost their individual contours, producing a homogeneous mass. In many sections the thrombi were all of a uniform homogeneous appearance with none of the structural components visible (Fig. 1).

As a rule, in either the partial or the complete vascular occlusions, only a few fine fibrils of fibrin were visible within the thrombus when only the routine hematoxylin-phloxine stain was used. However, with special staining a rather rich network of fibrin was usually demonstrated throughout the thrombus (Fig. 2).

Hemorrhagic lesions were very mild and infrequent. Sparse erythrocytes were seen in the perivascular spaces of only a small number of scattered vessels. An extravasation of blood sufficient to produce a notable filling of the Virchow-Robin space was very unusual. Although a mild, patchy, subarachnoid hemorrhage was common, no more than a few erythrocytes were observed within the leptomeninges. The walls of the cerebral vessel showed no degenerative or proliferative changes and displayed no involvement by inflammatory cells. A microglial reaction did not occur.

In general, the ganglion cells appeared normal but occasionally a few neurons revealed a mild swelling and chromatolysis. The myelin was essentially normal, showing only a very minimal perivascular degeneration around a few of the thrombosed vessels. There was no astrocytic proliferation.

#### *Moderately Severe Encephalitis*

In the more involved cases, thrombosis became even more extensive, implicating a relatively large percentage of the cerebral vessels of all

sizes, with either partial or complete occlusion of their lumina. The structure of most thrombi was essentially the same as that described in the milder cases except that more fibrin was usually present. Very few leukocytes were found in the thrombi. In the occlusions of the larger vessels, prominent collections of platelets were often detected within the thrombi, producing a somewhat lamellated appearance. In these larger vessels a multiplicity of branching threads of fibrin were frequently visible without the use of special stains. There was no organization of thrombotic material.

Perivascular hemorrhages became numerous. Many of these extended into the neural tissue in the vicinity of the perivascular spaces, producing ball petechiae. A small number of ring hemorrhages also developed. Diffuse intracerebral bleeding did not occur. A thick layer of blood might be found distending the subarachnoid space over large regions of the brain surface. A sparse, secondary, leukocytic exudate might occasionally be associated with the subarachnoid bleeding.

There were no changes in the blood vessel walls to account for the thrombosis and hemorrhage. In one of our cases proliferative changes occurred in the walls of several vessels in the region of the basal nuclei. Generally a few leukocytes, consisting of a mixture of polymorphonuclear neutrophils and mononuclear cells, were scattered irregularly around the adventitia of a small number of vessels (Fig. 3).

Many nerve cells throughout the gray matter of the brain revealed a marked tigrolysis and often a concomitant alteration of the cell nucleus. Only a few ghost cells resulted.

Mild alterations of the myelin sheaths were observed. The most characteristic involvement consisted of a narrow perivascular zone of swelling and a mild rarefaction of the myelin sheaths. Discrete perivascular foci of advanced demyelination were rare (Fig. 4). A few of these lesions occurred in only one case in this series. Usually there were a few areas of diffuse demyelination within the cerebral white substance. In some of the regions of myelin degeneration, a slight proliferation of the microglia occurred. This microglial reaction consisted principally of small microglial cells intermixed with a small number of red cells and fat-granule elements. A few scavenger cells were occasionally found in the adventitia of certain vessels infiltrated by leukocytes. The macroglia showed no evidence of activity.

#### *Very Severe Encephalitis*

The advanced degree of cerebral alteration occurred in only rare instances of pneumonic encephalitis. In our material only one case revealed this severe form. Thrombus formation was still the most striking pathologic finding. An even greater number of vessels of all

sizes were thrombosed. Many of the thrombi were similar to those previously described. However, a multiplicity of small vessels were filled with eosinophilic staining homogeneous material which resembled fresh platelet thrombi. Special stains, however, demonstrated that the thrombotic substance was comprised largely of coils of very coarse fibrin which tended to fuse into an almost solid mass (Fig. 5). In an occasional instance, fine fibers of fibrin extended outside the vessel to invade the perivascular parenchyma (Fig. 5).

Perivascular hemorrhages became more numerous and were of both the ball and ring types (Fig. 6). These perivascular extravasates frequently encircled small thrombosed vessels but the central thrombus was usually visible in only the ring petechiae, since the extravasated blood itself obscured the central vessel of the ball hemorrhage (Fig. 6). Prominent focal collections of mixtures of polymorphonuclear and mononuclear leukocytes were found in the centers of a small number of petechiae of both the ball and ring types. A few scavenger cells might occasionally be intermixed with the leukocytes.

Neurocellular alterations were very frequent and quite severe throughout the brain. The most striking finding consisted of marked shrinkage, pyknosis, and irregularity of the cell bodies of many of the neurons. Some cells were slightly swollen and stained very lightly to appear as ghost cells. A few were completely destroyed. In the deeper layers of the cortex there frequently occurred a disturbance of the polarity and lamination of the neurons, resulting in a wind-blown appearance of the involved areas (Fig. 7).

The myelin showed a more severe degenerative change. Small patchy areas of swollen and rarefied myelin sheaths were numerous, especially in the subcortical white matter and the centrum ovale (Fig. 8). A more marked and diffuse demyelination sometimes occurred in the vicinity of a large collection of petechiae. In these situations vacuolization and partial fragmentation of the neural tissue occasionally took place. Even in the areas of more severe myelin degeneration, only mild microglial proliferation occurred, associated with very few gitter or scavenger cells. The axis cylinders showed very little alteration even in these cases with severe involvement, the only exception being the destruction of axons in the areas directly involved by focal hemorrhage. A notable degree of astrocytic proliferation did not occur.

#### DISCUSSION

A very important result of this investigation was the observation that essentially identical pathologic lesions of the nervous system occur in all cases of pneumonic encephalitis, even though the etiologic

agent of the pneumonia varies greatly from case to case. The earliest encephalitic alterations consisted of diffuse congestion and thrombosis of a large number of the smaller cerebral vessels. Mild perivascular and subarachnoid bleeding soon developed. There was very little cellular inflammation, myelin degeneration, or nerve cell change.

In the more severe encephalitic complications many thrombosed vessels and hemorrhagic lesions were observed. The latter consisted of ring and ball petechiae as well as abundant subarachnoid bleeding. The thrombi involved many of the larger vessels as well as a majority of the smaller ones, and most of them contained abundant fibrin. Perivascular inflammatory elements were also somewhat more frequent. Nerve cell disease was moderately severe throughout the brain, resulting in occasional areas of neuronal devastation. Myelin sheath changes were more prominent but tended to remain quite mild. Although we have not had the opportunity to study the pathologic changes within the nervous system in the more chronic cases, we have been able to follow several patients clinically for more than a year. The fact that the symptomatology seemed progressive for many months suggested the presence of a very extensive involvement of the brain. Such a deduction, however, must be proved by future investigations.

The actual causative agent of the encephalitis has not been identified. Many theories are available which might be used in explaining this form of cerebral complication. These include: (1) pyrexia, (2) toxemia secondary to the pneumonia, (3) direct invasion of the brain by the bacterial agent causing the pneumonitis, (4) generalized virus infection involving the brain as well as the lungs, (5) activation of a latent filterable virus previously lying dormant within the nervous system, (6) drug hypersensitivity in cases treated with serum or sulfonamides, (7) anoxemia, and (8) allergic reaction.

The observation that encephalitis may develop in mild, afebrile cases, showing no signs of any notable degree of toxemia, and in patients already convalescing from their pneumonia seems sufficient to exclude pyrexia and toxemia as the causative factors. The paucity of inflammatory elements speaks against a direct bacterial invasion of the nervous system. Ingleby<sup>25</sup> found numerous inclusion bodies within the brain, lungs, and other viscera in cases of pneumonic encephalitis. She, therefore, postulated a virus as the causal factor for her cases. However, in other cases of encephalitis complicating the typical virus pneumonia, inclusion bodies have not been found (Perrone and Wright<sup>26</sup> and Golden<sup>24</sup>). Moreover, the pathologic changes within the central nervous system show no resemblance to those lesions described in the usual virus form of encephalitis.

Many of the so-called postinfectious encephalitides have been attributed to the activation of a latent neurotropic virus. The constantly uniform pathologic manifestations of pneumonic encephalitis make it necessary for one to consider this theory carefully. However, instead of the usual foci of perivascular demyelination observed in the postinfectious encephalitides, the pneumonic complication is characterized by thrombosis of many vessels and scattered hemorrhagic lesions, thereby disproving the possibility of any etiologic similarity between these two conditions.

A drug sensitivity has been implicated as the cause of certain cases of periarteritis nodosa and has been verified experimentally for the sulfonamide drugs by Rich and Rich and Gregory.<sup>20</sup> None of our patients were given serum and in the few who received sulfonamide drugs there was no apparent relation between the administration of the drug and the onset of cerebral symptoms.

An allergic reaction to the primary infectious condition frequently has been suggested as the cause of different types of postinfectious encephalitis. Ferarro<sup>30</sup> produced cerebral lesions experimentally that were very similar to those of scarlatinal encephalitis. The fact that either foreign proteins or brain extracts of a heterologous animal were used as the antigens in his experiments casts doubt upon such a hypothesis as the explanation for the lesions observed in the present study. However, this allergic theory cannot be discarded without further investigation.

Golden<sup>24</sup> believed that the encephalitic involvement in cases of pneumonia might be the result of anoxemia. This concept seems very unlikely since encephalitis often occurs in cases without any clinical evidence of oxygen want and also after the pneumonitis has largely resolved. Since cyanosis and respiratory distress occur with only minimal lung involvement, it is possible that the symptoms suggesting anoxemia may actually be central rather than pulmonary in origin. The multiplicity of thrombosed cerebral vessels in pneumonic encephalitis should be adequate to differentiate this condition from anoxia.

The occurrence of uniformly identical cerebral alterations in all of our cases indicates that pneumonic encephalitis most likely has a constant pathogenesis and is not directly related to the etiologic agent of the pneumonia, which may be one of various bacteria, viruses, or even lipid. The only constant factor in these cerebral complications is the nonspecific inflammatory process within the lungs. Therefore, it seems logical to infer that in the pulmonary tissue itself may be found the primary cause of the encephalitis.

Since the most characteristic pathologic manifestation of this variety

of encephalitis is a very extensive thrombosis, we have felt that perhaps the basis of this illness may be related in some way to disturbance in the clotting mechanism. We would like to suggest further that some factor from the lung tissue itself may gain access to the circulation under pathologic conditions and thus initiate the abnormal intravascular clotting observed within the nervous system. Experiments now under way seem to substantiate this concept.

#### SUMMARY

1. A survey of the literature regarding pneumonic encephalitis reveals only a small number of sporadic clinical and pathologic case reports.
2. A careful study of the pathologic lesions of the brain in 10 cases of pneumonic encephalitis revealed that the cerebral alterations are uniform throughout the entire series, even though the cause of the pneumonitis is highly variable.
3. Extensive thrombosis and prominent perivascular hemorrhages are the outstanding microscopic findings observed in the nervous system.
4. Various theories regarding the pathogenesis of this type of encephalitis have been presented. The prodigious number of thrombosed cerebral vessels observed in this study suggests the possibility that some alteration in the clotting mechanism of the blood may cause these cerebral lesions.
5. The constancy of the cerebral lesions, regardless of the type of pneumonia, indicates that the real cause of the encephalitis may be the pulmonary tissue itself. Some factor from the lung parenchyma may possibly accelerate intravascular clotting.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

### PLATE 121

FIG. 1. There is a partial obliteration of the vessel lumen by pale homogeneous thrombotic material. Red blood cells continue to circulate through a narrowed channel. Hematoxylin-phloxine stain.  $\times 460$ .

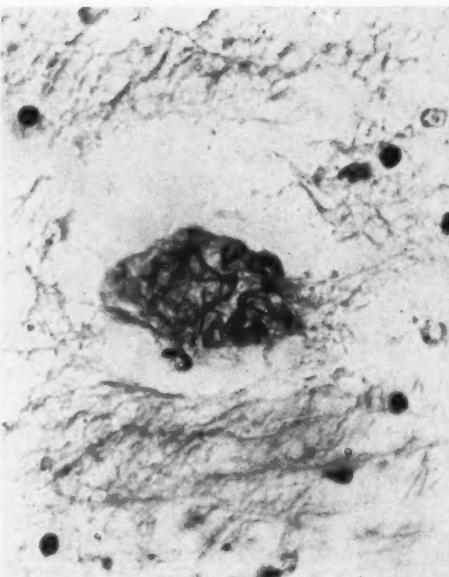
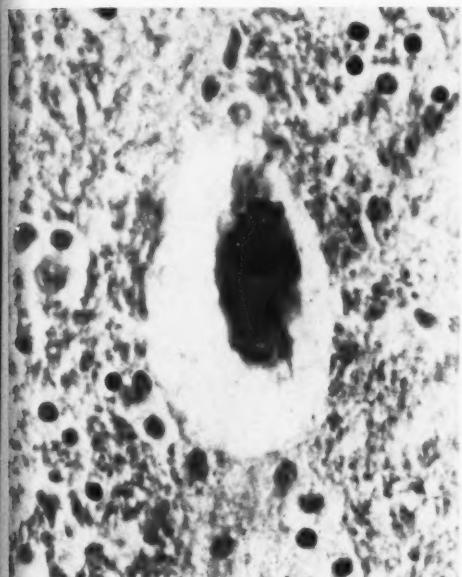
FIG. 2. The vascular lumen is filled with abundant fibrillar fibrin. Mild rarefaction and swelling of the myelin around the vessel is evident. Phosphotungstic acid hematoxylin stain.  $\times 460$ .

FIG. 3. Sparse collections of neutrophils and mononuclear leukocytes are scattered irregularly along the adventitia of the vessel. There are only very few microglial elements. Nissl stain.  $\times 460$ .

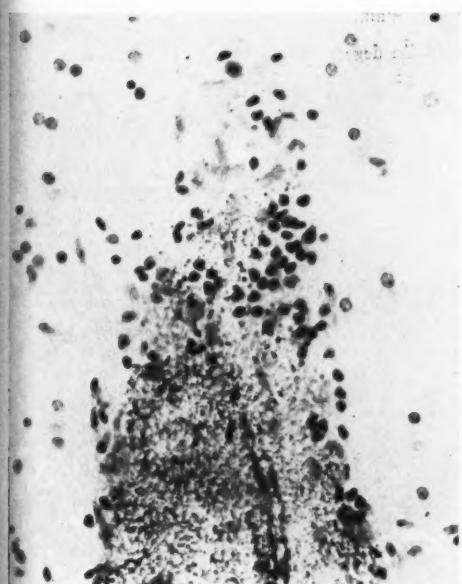
FIG. 4. This section reveals a prominent perivascular focus of demyelination. Hematoxylin-phloxine stain.  $\times 140$ .

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PLATE 122

FIG. 5. A ring petechial hemorrhage is illustrated. Coarse coils of fibrin occur within the central vessel with fine threads of fibrin extending into the necrotic perivascular parenchyma. Phosphotungstic acid hematoxylin stain.  $\times 460$ .

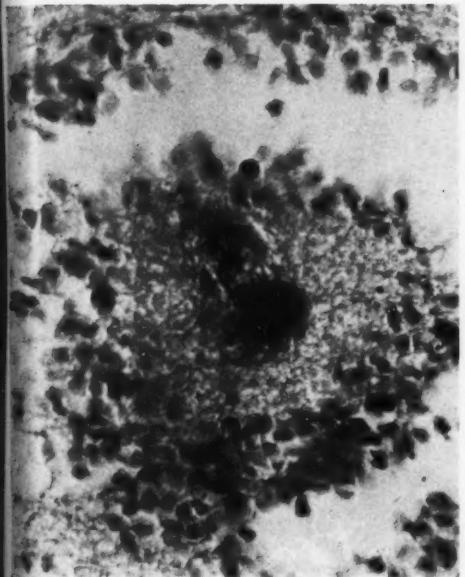
FIG. 6. This section reveals three ring and one ball type of hemorrhage. There is a thrombus in the center of each ring hemorrhage. Weil stain.  $\times 45$ .

FIG. 7. Marked changes in the nerve cells are found in this area. Most of the neurons are shrunken and pyknotic. There are a few ghost cells. The polarity of most of the neurons is disturbed. Nissl stain.  $\times 230$ .

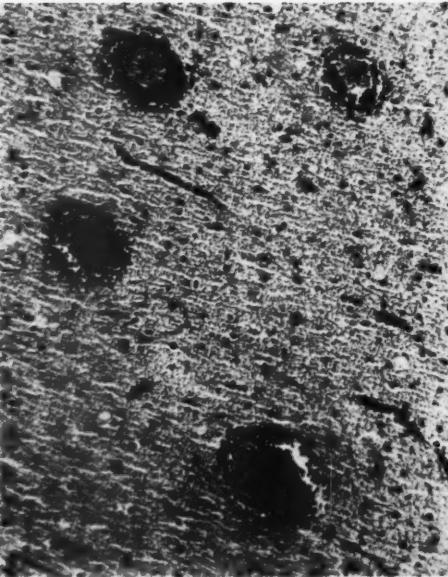
FIG. 8. Most of the tissue has undergone myelin degeneration. Many of the focal lesions are perivascular. Weil stain.  $\times 140$ .



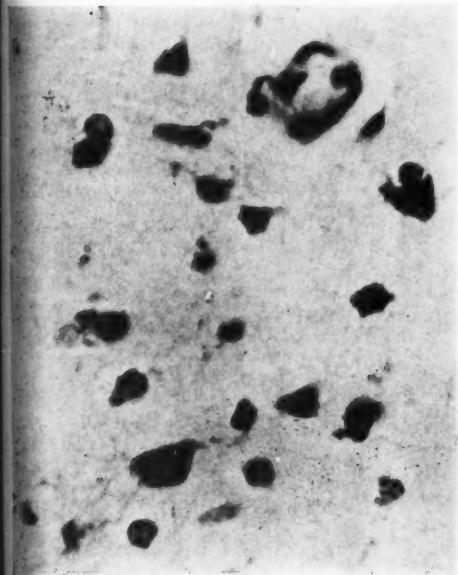




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## ADENOMATOID TRANSFORMATION OF THE GLOMERULAR CAPSULAR EPITHELIUM \*

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The following case is of interest because of a unique renal lesion which seems not to have been previously described.

The patient was an Italian-American housewife, 54 years old, admitted to the Presbyterian Hospital because of jaundice of 1 month's duration. In the past her health had been good, despite the fact that her diet had been low in meat and rich in its carbohydrate content. Her family history was irrelevant. Five months before admission she began to have eight to nine watery bowel movements daily. The stools were never bloody, tarry, or foul. One month later anorexia, swelling of the abdomen and ankles, and vague pain in the epigastrum and lower extremities appeared. For 1 month prior to entry the patient had had jaundice, with dark urine, light stools, nausea and occasional vomiting. A pronounced weight loss also had occurred.

On physical examination the patient appeared obese, jaundiced, and chronically ill. Examination of the heart and lungs was negative save for an apical systolic murmur. The liver edge extended to the pelvic brim and was hard, nodular, and not tender. No ascites was evident, but pitting edema of the lower extremities was pronounced.

The laboratory data of interest follow. Urine: specific gravity, 1.005 to 1.018; albumin, 2 plus to 4 plus; glucose on five occasions, 0, once was 1 plus; bile, 3 plus; 5 to 10 white blood cells, rare red blood cells, occasional hyaline and granular casts. Blood: red blood cells, 4,020,000 per cmm.; hemoglobin, 11.6 gm. per cent; white blood cells, 28,000 per cmm. (polymorphonuclear cells, 84 per cent, slight shift to left); platelets, "slightly reduced"; erythrocyte sedimentation rate, 77 mm. per 1 hour; Kline test, negative. Blood serum: alkaline phosphatase, 26.0 Bodansky units per cent; urea nitrogen, 13 mg. per cent; bilirubin, 6.3 mg. per cent; cholesterol, 625 mg. per cent; albumin, 2.8 gm. per cent; globulin, 3.6 gm. per cent; euglobulin, 0.6 gm. per cent; cephalin flocculation, negative; fasting sugar, 89 mg. per cent. Prothrombin time, 26.4 seconds. Stool: guaiac test, 4 plus. Urine: *Streptococcus viridans* and *Staphylococcus albus* obtained by culture. Electrocardiogram, sinus tachycardia. Roentgenogram, suggestion of esophageal varices.

The course was afebrile but her condition grew worse steadily. Obstructive jaundice persisted and there was leukocytosis ranging as high as 42,800 white blood cells per cmm. Finally the patient became stuporous and died on the 39th day following her admission to the hospital.

At necropsy the body was that of a short, obese, deeply jaundiced, middle-aged white female with a moderate amount of pitting edema over the sacrum, abdominal wall, left breast, and both lower extremities. About 250 cc. of thin, faintly turbid, pale, yellow fluid was present in the peritoneal cavity and smaller amounts of similar fluid were found in the pleural cavities. Firm fibrous adhesions bound part of the omentum to the abdominal wall but otherwise the serous surfaces were smooth and glistening. Briefly, the abnormal anatomical find-

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ings were as follows: chronic cholecystitis; calculi in gallbladder; carcinoma of gallbladder with extension to common bile duct and to liver; secondary carcinoma in lymph node (hepatic), liver, and lung; jaundice; ascites; varices of esophageal veins due to portal obstruction (by neoplastic tissue); splenomegaly; varices of hemorrhoidal veins; extramedullary hematopoiesis in spleen, liver, periadrenal adipose tissue, kidneys, and stomach; ulcer of stomach (acute); cardiac hypertrophy; chronic cervicitis; polyps of cervix and of uterus; fibrous peritoneal adhesions; fibroma of left kidney; hypertrophy of kidneys and proliferation of capsular and tubular epithelium (described below).

The kidneys were alike. Each was considerably enlarged, the right weighing 320 gm. and the left, 300 gm. The capsule stripped readily, revealing a surface which was normal except for the presence of a few shallow scars. On section, the renal tissue was moderately jaundiced. Corticomedullary differentiation was distinct and all markings appeared normal. In a centrally placed pyramid on the left there was a small fibroma. The pelvis, calyces, and ureters were normal.

Histological examination revealed that in both kidneys nearly all glomeruli had undergone a striking change. Their parietal capsular epithelium was replaced by a layer of tall columnar cells with oval, hyperchromatic nuclei arranged with their long axes perpendicular to the basement membrane. This peculiar epithelium was generally one cell thick, but in many places, especially near the exit of the proximal convoluted tubules, it was two or even three cells in depth. Occasionally, small plications projected into the capsular space. The cells were quite uniform in size and in shape, but as they approached the reflection onto the glomerular tuft they gradually became less columnar, and the glomerular tufts were covered by the usual flattened squamous "visceral" epithelium. No mitotic figures were seen and nowhere did these proliferating cells invade beyond the basement membrane into the surrounding parenchyma. There was, however, an occasional extension, for a short distance only, into some of the proximal convoluted tubules. Spherical bodies, staining red with the Laidlaw stain for inclusion bodies, were found within the nuclei of a few of the proliferating capsular cells. They were also demonstrated within the nuclei of the proximal convoluted tubular epithelium, but since similar bodies could occasionally be found in control material their significance is questionable. The capsular spaces were generally clear, but in some glomeruli there were curious hyaline, refractile bodies, some round, others oval, and about many of them infolded hyperchromatic capsular cells were arranged in the form of rosettes. In tissue fixed with

Zenker's fluid these hyaline glomerular bodies were eosinophilic with hematoxylin and eosin, but purple-black with the azan carmine-Wilder stain. In all formalin-fixed tissue they were purple-black. The von Kossa stain revealed that they were not composed of calcium salts, and with the Laidlaw inclusion-body stain they were a pale brown. The glomerular tufts and their associated visceral epithelial cells were occasionally compressed and relatively bloodless. In the few glomeruli that appeared to escape this change it was usually possible to detect a few cells in the capsule that were thickened and hyperchromatic. Other portions of the nephron appeared normal and the only vascular abnormality was minimal arteriolar sclerosis. In the interstitial tissue of the medulla and in the adipose tissue of the pelvis a moderate infiltration with hematopoietic tissue was found.

The gallbladder showed changes consistent with the diagnosis of chronic cholecystitis and at one point its mucosa was replaced by neoplastic tissue. The neoplasm here, as well as elsewhere, was a poorly differentiated, largely necrotic adenocarcinoma, not producing mucin. Metastases were found in the liver, lungs, and regional lymph nodes. None were found in the kidneys. Although no metastases were found in the lumbar vertebrae or sternum and although the patient did not have a profound anemia, intensive extramedullary hematopoiesis was found in the spleen, liver, adrenal, stomach, as well as the kidneys.

#### COMMENT

The significance of the described alteration in Bowman's capsule is obscure. It appears to be a diffuse neoplastic change, probably primary in the kidney. A renal primary is indicated by two features. One, the cytology of the abnormal capsular epithelium differs from that of the biliary tract tumor; and two, no route for metastasis to the space of Bowman is apparent since no tumor cells are found within the glomerular tufts.

Changes roughly resembling those herein described have been noted by others in kidneys which were the sites of other primary or secondary tumors. Thus Lauterburg's<sup>1</sup> report contains a sketch of a glomerulus superficially much like that just described, but differing in two respects. First, the tumor cells lining the capsule were identical cytologically with those of a primary bronchogenic carcinoma which had metastasized extensively to the kidney. Second, only few glomeruli were involved, and the author believed that the abnormal cells were derived from localized metastatic tumor nodules, possibly extending along tubules which acted as conduits. In a peculiar case of multicentric primary carcinoma of the kidney reported by Lisa,<sup>2</sup> a single

glomerulus in a single section showed similar replacement of the capsular epithelium by a layer of tumor cells, and in addition a tumor cell was noted in the glomerular basement membrane. The latter finding together with the restriction of the change to a single glomerulus clearly distinguishes it from the changes just described. The resemblance of the diffuse capsular epithelial transformation to pulmonary adenomatosis of men and sheep (*jaagsiekte*)<sup>3</sup> is rather striking, but of obscure significance. Extension of proximal convoluted tubular conical cells along the glomerular parietal basement membrane has been noted in human kidneys, and is of frequent occurrence in mice,<sup>4</sup> but its resemblance to the lesion here described is too remote to suggest that the latter change is of the same nature.

If then, as appears likely, the capsular transformation found in this case represents a primary neoplastic change, the possibility that it follows the urinary excretion of some growth-inciting agent, virus, or chemical may be raised. Unfortunately, there seems, at present, no way of obtaining further information as to the cause of this unusual lesion.

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#### DESCRIPTION OF PLATE

##### PLATE 123

FIG. 1. Kidney, showing adenomatoid transformation of capsular epithelium with occasional extension into proximal convoluted tubules. Hematoxylin and eosin stain.  $\times 95$ .

FIG. 2. Drawing of a glomerulus illustrating the chief points of the lesion. Of note are the plications, rosette formation, absence of invasion, sparing of glomerular tuft, and extension into proximal convoluted tubule. Hematoxylin and eosin stain.  $\times 215$ .





1

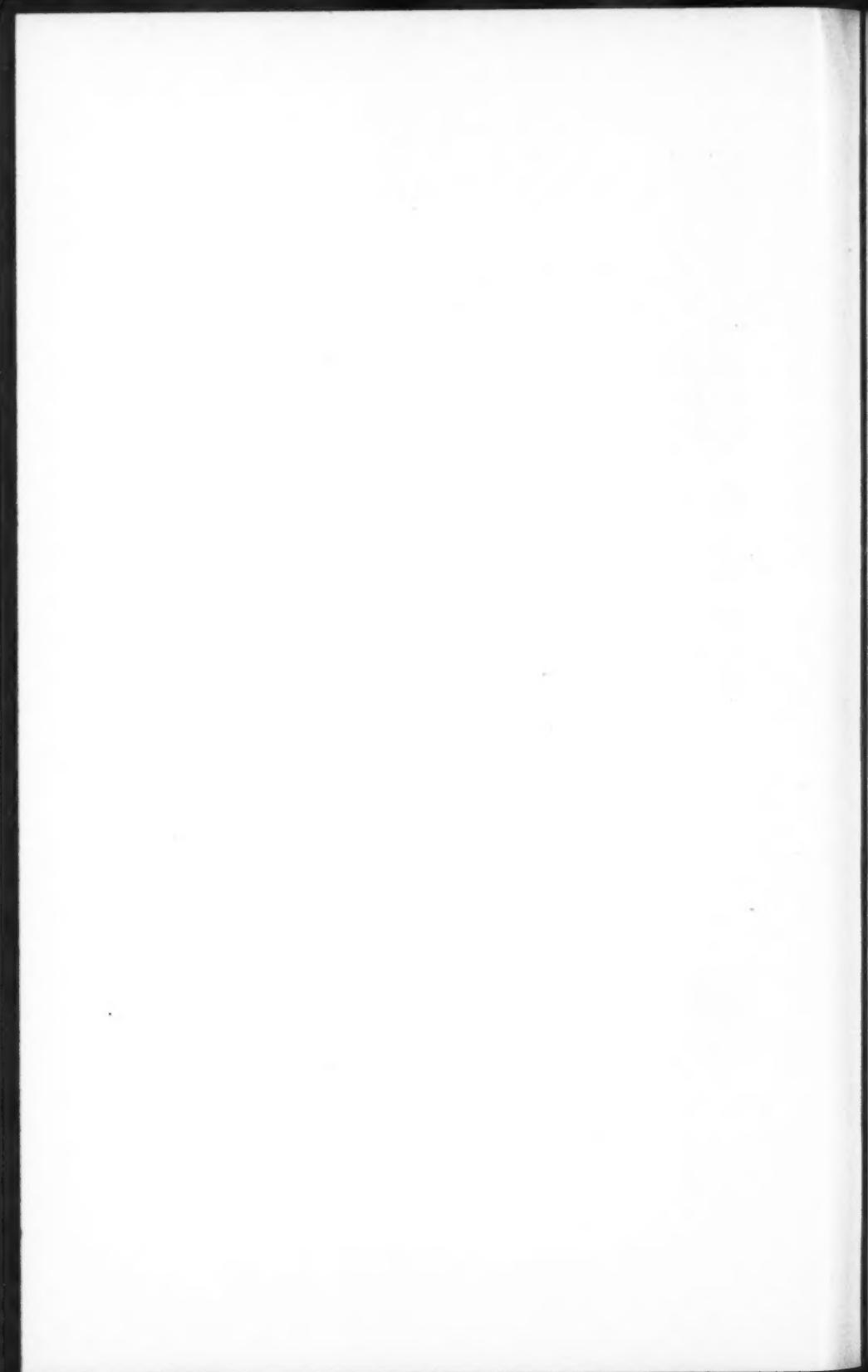


2



Eisen

Glomerular Capsular Epithelium



BISMUTH PIGMENTATION  
ITS HISTOCHEMICAL IDENTIFICATION \*

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On occasion it is desirable to differentiate the pigmentation produced by compounds of bismuth from those due to other causes. Pigmentary deposits containing bismuth have been described as a diffuse, dark discoloration of the colon, beginning sharply at the ileocecal valve and involving the mucosa in varying degrees.<sup>1-7</sup> Bismuth is one of the several metals which may be deposited in the gingiva or other areas of the oral mucosa and induce brown, bluish, or black discoloration.<sup>8,9</sup> Deposition of black bismuth sulfide has also been described in the vagina<sup>7,10,11</sup> and in the bladder.<sup>12,13</sup> Deep pigmentation of the skin due to bismuth deposition may occur in exceptionally rare cases.<sup>4,14,15</sup>

In the present investigation, Castel's method<sup>16</sup> for the histochemical identification of bismuth was adapted for the demonstration of bismuth sulfide in tissue sections and in gross specimens. Thus, a simple histochemical procedure for the identification of bismuth, deposited as black sulfide as it may occur in surgical or autopsy material, is available. It was found that bismuth pigmentation of the large intestines occurs fairly frequently. Among the last 340 autopsies performed at the Mount Sinai Hospital, 4 examples were encountered.

METHOD

The method depends on the property of hydrogen peroxide of decolorizing bismuth sulfide instantaneously. Black bismuth sulfide is thus transformed into white bismuth sulfate.<sup>17</sup> By then treating the sections with a slightly modified Castel reagent,<sup>16</sup> containing brucine sulfate and potassium iodide, the bismuth sulfate is transformed into an orange-red deposit. This reaction, based on the work of Léger,<sup>18</sup> depends on the fact that numerous organic bases form insoluble double iodides with bismuth of the general formula  $BI_3 \cdot B \cdot HI$ , in which B represents the base.<sup>19</sup>

In this study, frozen as well as paraffin sections were used. Paraffin sections were deparaffinized in the usual manner and were then treated with a few drops of superoxol (30 per cent hydrogen peroxide, Merck). This reagent is best kept in a dark bottle in the refrigerator. The black color of bismuth sulfide disappears in a few seconds. Sections

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are then washed thoroughly in tap water and placed in a Coplin jar, containing the modified Castel reagent.<sup>10</sup> The latter is made by dissolving 0.25 gm. of brucine sulfate (Merck or Eastman Kodak) in 100 cc. of distilled water containing 2 or 3 drops of concentrated sulfuric acid. After the brucine sulfate has dissolved, 2 gm. of potassium iodide are added. The reagent is kept in a brown bottle and filtered before use. After 1 hour, the sections are put into another jar containing the brucine reagent diluted with three parts of distilled water, and gently shaken in order to remove precipitates. Most of the remaining fluid is removed by gentle blotting and the slide covered with levulose solution (prepared by dissolving 30 gm. of levulose in 20 cc. of water by heating to 37° C. for 24 hours), to which a drop of the diluted Castel reagent is added with a glass rod or toothpick. If a counterstain is desired, the slide is stained for 4 minutes with a mixture containing 100 cc. of the nondiluted brucine sulfate reagent and 1 cc. of a 1 per cent aqueous solution of light green SF (Hartman-Leddon Co.), filtered before use. The stain will keep well although the orange color may darken to some degree.

This method can be applied also to gross specimens. Fixed material is preferable, but fresh tissues are also suitable. The concentrated hydrogen peroxide solution is added drop by drop to the area under investigation. Decolorization will take place immediately if bismuth sulfide is present. The specimen is then thoroughly washed in running water for several minutes to remove excess hydrogen peroxide. The modified Castel reagent is then applied to this surface. An intense orange precipitate is formed, giving the same color to the mucosa.

#### CASES WITH BISMUTH PIGMENTATION

##### *Case 1*

A white male, 41 years old, was admitted to the hospital because of dyspnea, generalized edema, and severe anemia. Physical examination revealed marked enlargement of heart, liver, and spleen. Petechiae were found on the conjunctivae. A positive Wassermann reaction of the blood was present. For this reason the patient was given biweekly bismuth injections. He underwent a splenectomy for intractable anemia with good results, and was discharged after 4 months. Following this he continued to receive weekly bismuth injections. Five months later he was readmitted in cardiac failure, and died after 14 days in the hospital.

The pertinent post-mortem findings (autopsy No. 12768) were cerebral hemorrhage, healing stage of subacute bacterial endocarditis upon chronic rheumatic mitral and aortic valvular disease, and subacute glomerulonephritis. Patchy gray and black areas of discoloration were found in the cecum. In the tonsils similar pigmentation was visible in the crypts. Microscopic sections through the discolored areas

of the cecum showed black pigment in the walls of the most superficial capillaries. Characteristically, this pigment was found in the capillary endothelium, producing a singly or doubly contoured black line. Very little pigment was seen elsewhere. Sections through the tonsils showed lymphatic tissue covered by one layer of cylindrical epithelium frequently arranged in a papillary fashion. The subepithelial capillary walls showed marked impregnation by a dark pigment (Fig. 1). The intensity of this reaction varied in different vessels, so much so that in some the lumen was obscured by the very heavy deposit. Moreover, black pigment was seen also in some histiocytes as well as free in the submucosa, without definite relation to cellular structures.

#### *Case 2*

A white female, 30 years old, had a history of rheumatic fever and chorea in childhood. She had been suffering from mild dyspnea for the past 5 years. Two years before admission hypertension was noted. Wassermann reaction of the blood was 4 plus and therefore she was treated elsewhere with weekly bismuth injections for 1 year. Because of sudden severe dyspnea and cyanosis she was admitted to the Mount Sinai Hospital, where her blood pressure was found to be 250/140 mm. Hg. Urine examination revealed 3 plus albumin, hyaline and granular casts, and occasional red blood cells. She died 2 days later.

The pertinent post-mortem findings (autopsy No. 12933) were rheumatic heart disease and malignant nephrosclerosis. The cecum and ascending colon showed a very marked, diffuse, black discoloration. On microscopic study, the findings in regard to the location of pigment were exactly as in the previous case. Pigment-laden histiocytes and extracellular granules in the mucosa were, however, quite prominent. Again, no pigment was seen in the deeper layers (Fig. 2).

#### *Case 3*

A white male, 65 years old, gave a history of chancre 25 years before admission to the hospital. Eight months before, his blood and spinal fluid had given a positive Wassermann reaction and a paretic colloidal gold curve. He was then treated with fever therapy, arsphenamine, and two injections of 1 cc. of bismuth preparation in oil. Five weeks before admission, a gastrostomy was performed elsewhere because of carcinoma of the esophagus. The patient entered Mount Sinai Hospital for resection, but died within a few days and before operation.

The most important post-mortem findings (autopsy No. 12952) were squamous cell carcinoma of the esophagus with metastases to the lungs and lymph nodes, anthracosilicosis, pulmonary tuberculosis with recent bronchogenic spread, and syphilitic mesaortitis. The mucosa of the cecum and ascending colon was diffusely brown. Microscopic sections through this area showed only occasional superficial capillaries impregnated with a dark pigment. Fine pigment granules were seen in the endothelial cells of less involved capillaries, and in an occasional histiocyte in the mucosa.

*Case 4*

A white male, 55 years old, had a history of chancre 34 years prior to admission and of treatment for 7 years with injections of bismuth and arsphenamine. Three years before admission examination of his blood revealed a 1 plus Wassermann reaction. He was treated for another year with bismuth and arsphenamine. Thereafter, his serologic tests became normal. A few months later he acquired a second syphilitic infection, proved by dark-field examination. Again he was treated with bismuth and arsphenamine parenterally. The patient was admitted to the hospital because of vomiting, severe headache, and mental confusion. His blood pressure was 250/110 mg. Hg and blood urea nitrogen, 150 mg. per cent. The patient died 3 days later.

The significant findings at the post-mortem examination (autopsy No. 13022) were malignant nephrosclerosis with marked cardiac hypertrophy and dilatation. The large intestine showed diffuse black pigmentation of the mucosa, especially severe in the cecum, starting sharply at the ileocecal valve and diminishing in intensity distally. A pigment line was visible on the upper gingival margins. Microscopic sections of the colon showed, as before, impregnation of many of the capillary walls by black pigment, and altogether a similar histologic picture. Sections through the gingiva revealed pigment deposited in superficial capillaries and histiocytes, as well as severe chronic and acute inflammation with superficial ulceration.

*Case 5*

A white male, 59 years old, had had a generalized rash, including palms and soles, at the age of 26. He was treated with mercury inunctions. Thirteen years later examination of his blood revealed a 4 plus Wassermann reaction. His spinal fluid was negative at that time. For the following 3 years he was treated continuously with bismuth and salvarsan injections. Examination of his blood 2 years prior to admission revealed a positive Wassermann reaction. He was given a course of eighteen bismuth and arsphenamine injections, following which the Wassermann reaction of the blood became negative. On one occasion, when the patient visited the Out Patient Department complaining of a swelling in his mouth, physical examination revealed a small growth extruding between the third and fourth left lower teeth. No mention was made of a black discoloration of the gingiva.

A specimen of this area, taken for biopsy (surgical specimen No. 81006), showed marked acute and chronic inflammation. Except for a greater intensity, the gingiva here revealed pigment deposition similar to that seen in the preceding case.

**COMMENT**

Microscopically, the outstanding feature of the deposition of bismuth is the impregnation of the capillary walls, predominantly in the superficial layers of the mucosa. When little is present, it is found only here; when much pigment is deposited, bismuth sulfide is seen also in histiocytes and free in tissue spaces. It is never encountered

in the deeper layers. This behavior is identical in the mucous membrane of the mouth and in that of the large intestine. In all cases in which bismuth sulfide was seen, it could be identified easily with the above-described method.

Among the pigments occurring in the oral cavity, melanin and iron are easily distinguished by their location. Iron, which may be present as the black sulfide, is oxidized by concentrated hydrogen peroxide and transformed into the usual golden brown hemosiderin. It does not react with Castel's reagent<sup>16</sup> but gives the typical iron reactions. Melanin is not bleached by a short treatment with hydrogen peroxide and does not give a reaction with the brucine reagent. Among the exogenous pigments, lead sulfide is the most important to exclude. Since it is deposited in vessel walls in the same manner as bismuth, it cannot be differentiated in routine sections. In order to test the bismuth reagent on lead sulfide in tissue sections, slides prepared according to Gomori's method<sup>20</sup> for the demonstration of acid phosphatase were used. By this technic, the phosphatase activity is demonstrated by the deposition of black lead sulfide. Preparations of this kind were oxidized with hydrogen peroxide. The lead sulfide is immediately discolored in the same fashion as bismuth sulfide, the sulfate being formed. However, treatment with Castel's reagent brings about only a slightly yellowish tinge of the lead deposits ( $PbI_2$ ), in contrast to the brilliant orange-red formed with bismuth.

Copper, silver, and mercury may also be deposited in capillary walls. No tissue material was available for study. However, in the test tube, as already shown by Castel,<sup>16</sup> silver and mercury give yellow, and copper gives brown precipitates, thus differing from bismuth. All these metals react with the iodide but do not combine with the brucine to form double salts as bismuth does.<sup>19</sup>

In the large intestine, the most frequent cause of dark pigmentation is "melanosis." In this condition, a brown granular pigment is deposited in the histiocytes of the lamina propria and occasionally in the submucosa. This pigment has been grouped with the melanins.<sup>21, 22</sup> It is usually found in persons suffering from constipation. It has been shown that its occurrence is dependent upon the intake of the emodin-bearing group of cathartics (cascara, aloes, frangula, Rheum, and senna).<sup>23, 24</sup> On microscopic examination, a distinction between bismuth and this pigment is readily made, since the latter never impregnates the capillary walls. An immediate distinction can be made at autopsy by applying the previously described gross reaction to fresh tissue. This melanin is not discolored by the short application of hydrogen peroxide and does not give the reaction with Castel's reagent.<sup>16</sup>

A second specific test, also based on Léger's work,<sup>18</sup> is available for identification of bismuth in tissue sections. Quinine sulfate and potassium iodide give a yellow precipitate with bismuth. Komaya,<sup>25</sup> among others,<sup>4</sup> adapted this reaction for the use in tissue sections. While this method gives good results in frozen sections, we found Castel's reagent<sup>16</sup> much more reliable and simpler for use in paraffin-embedded material.

No attempt was made to apply the specific bismuth stain systematically to all organs. However, on applying the stain to the kidney sections of cases 2 and 4, as well as of another case not included in this series, it showed refractile globules in the epithelium of the proximal convoluted tubules, as described by Pappenheimer and Maechling.<sup>26</sup> These authors studied the staining properties of these inclusion bodies extensively. They found them to react regularly with the Weigert-Spielmeyer stain. They became dark on treatment with hydrogen or ammonium sulfide, but did not give the more specific histochemical reactions for bismuth with stannous chloride-sodium hydroxide and with Komaya's reagent.<sup>25</sup> Apparently, similar bodies were described also by Langhans<sup>27</sup> in tissue sections of experimental animals, and in epithelial cells of human urinary sediments by others.<sup>28-32</sup> In our material most of these inclusion bodies reacted with Castel's reagent.<sup>16</sup> The majority gave a distinct orange color, although some stained yellow and others remained unstained. Desquamated epithelial cells and casts gave an occasional positive reaction.

To our knowledge no statistics are available concerning the incidence of bismuth pigmentation in the colon. Wiener<sup>11</sup> and Heyman<sup>7</sup> stated that deposition of bismuth is of very rare occurrence. However, since special attention has been paid to proper identification of discolored areas in the mucosa of the large intestine, we have encountered this condition more frequently.

Case 3 is of special interest. Here bismuth pigmentation of the colon was found although only two injections of 1 cc. of a bismuth preparation were given. Grossly, "melanosis coli" was diagnosed. However, the pigment showed the typical microscopic appearance and histochemical behavior of bismuth sulfide. Because of this, inquiries were made which revealed that the patient had received this small amount of bismuth at another hospital. The amount of histologically demonstrable bismuth varied markedly in the other three patients, although all of them had received intensive treatment. This is in full agreement with the findings of Sollmann, Cole, and Henderson<sup>33</sup> who determined the bismuth content of various organs of patients who had received bismuth. In 23 cases the colon contained between 0.025 and

3.0 mg. of bismuth in 100 gm. of wet tissue. The average was 0.115 mg. per 100 gm. of wet tissue. They found that the colon ranked fifth in bismuth content, after kidney, liver, spleen, and bile. In one case, described previously by one of us,<sup>6</sup> in which bismuth therapy led to fatal intoxication, 5.8 mg. of bismuth per 100 gm. of wet tissue was found in the colon.

#### SUMMARY

Castel's method for the demonstration of bismuth in tissue preparations was adapted for the identification of bismuth sulfide in frozen sections, paraffin sections, and in gross specimens. The method permits histochemical identification of bismuth sulfide pigmentation in any tissue. Bismuth discoloration of the colon is apparently not infrequent. It was found in four of 340 consecutive autopsies. Even small amounts of injected bismuth may lead to the deposition of histochemically demonstrable bismuth sulfide in the large bowel. The inclusion bodies found in the renal epithelial cells following the use of bismuth preparations frequently give a positive reaction with Castel's reagent.

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#### DESCRIPTION OF PLATE

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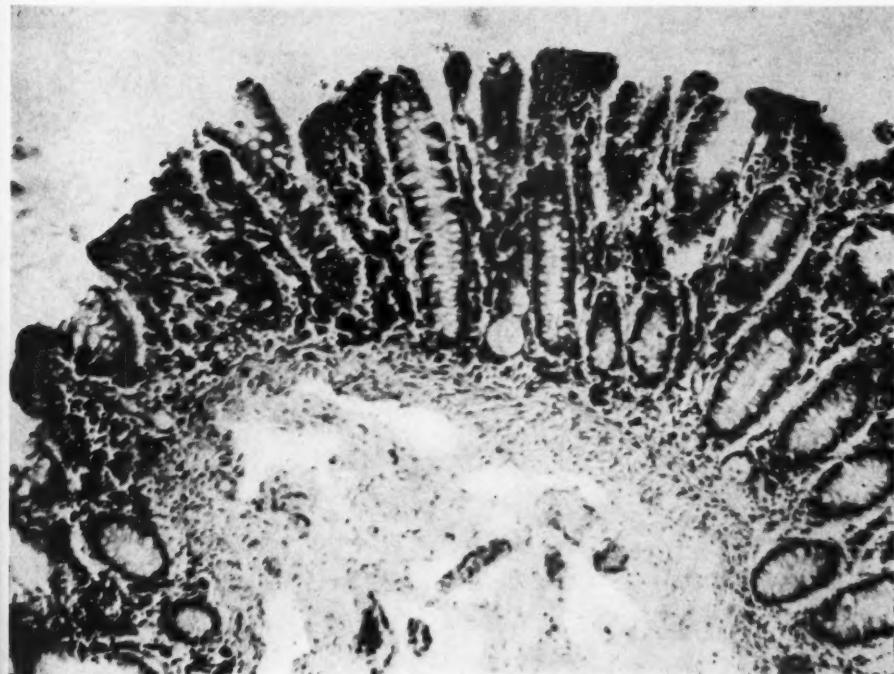
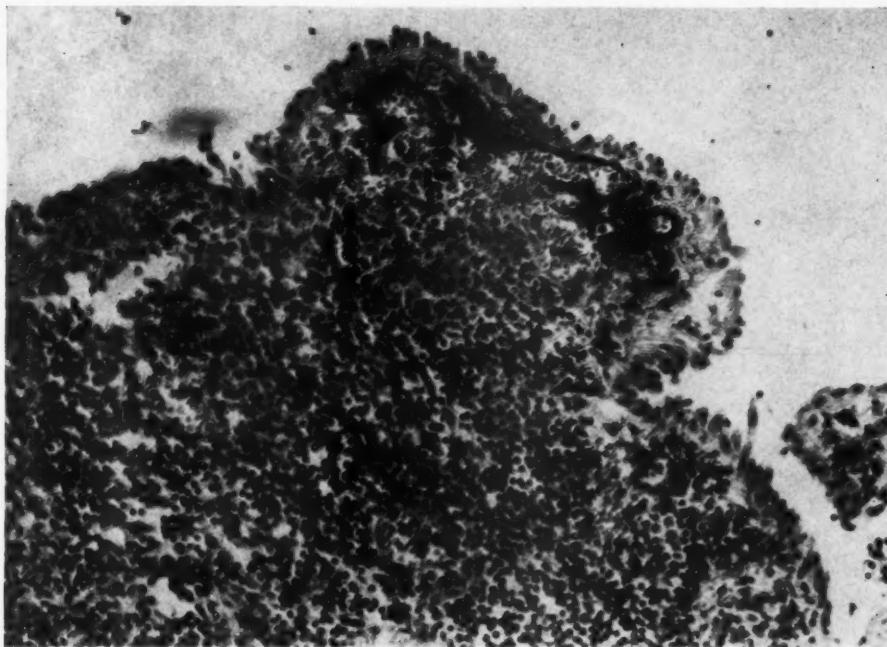
##### PLATE 124

FIG. 1. Case 1. Section through tonsil, showing superficial capillaries impregnated by black bismuth sulfide. Occasionally pigment is seen in histiocytes. Hematoxylin and eosin stain.  $\times 145$ .

FIG. 2. Case 2. Section through colon, showing extensive deposition of bismuth sulfide in mucosa. Hematoxylin and eosin stain.  $\times 70$ .

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Wachstein and Zak

Histochemical Identification of Bismuth



## OSSIFYING CARTILAGE AND THROMBI IN THE HEARTS OF RATS \*

EDMOND J. FARRIS, Ph.D., ELEANOR H. YEAKEL, Ph.D., and MARGARET M. SEITNER, M.A.  
(From The Wistar Institute of Anatomy and Biology, Philadelphia 4, Pa.)

Dissection and autopsies of rats in The Wistar Institute animal colony have disclosed two types of grossly visible pathologic lesions in the heart; namely, thrombi in the left atrium and ossifying cartilage in the left ventricle. Of gray Norway and Wistar albino rats 336 and 807 were examined, respectively, these rats being part of a colony established for studies in aging. A number were dissected after anesthetization to provide material for research on senescence, and the remainder were autopsied after dying from natural causes.

### THROMBOSIS

Thrombi were detected in the left atrium only. They were seen in rats of both strains after death from disease, and in gray Norways after anesthetization, when agonal thrombosis seemed unlikely. All of the thrombi were pale yellow, firm, and in many instances large enough to fill the chamber completely. Usually each was attached loosely to the atrial wall. Microscopic preparations of thrombi from 2 autopsied rats showed no signs of organization, resembling in this respect the thrombi described by Wilens and Sproul.<sup>1</sup> These authors remarked that fairly old rats (over 700 days of age) were particularly susceptible to the lesions. Although the average age of rats with cardiac thrombi autopsied by us was 773 days, thrombi were seen in gray rats dissected at 200 days (3 animals) and 400 days (2 rats) of age, and in an autopsied animal aged 229 days. The lesions were observed in a total of 24 gray Norway rats and 2 albinos, comprising 7 per cent of all grays examined, and considerably less than 1 per cent of all albinos.

### OSSIFYING CARTILAGE

Routinely the left ventricle was opened by a longitudinal incision, and the blood removed. Occasionally there was disclosed an ivory-colored, brittle structure clinging to the surface of the wall, varying in shape from a single roughened spicule to a coarse network with irregular branchings. Each structure was attached loosely to the endocardium and could be pulled away intact when sufficient traction was exerted upon it. The usual site was at or near the apex.

Two hearts containing prominent spicules were cleared and treated with alizarin-red-S. In both specimens the spicules stained a bright

\* Aided in part by a grant from the Samuel S. Fels Fund.

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red, indicating the presence of calcium. Figure 1 is an enlarged photograph of the cleared hearts, showing the gross structure of the calcified bodies.

Microscopic sections were made of 7 specimens, *in situ*. In most instances the structures were composed of cells resembling the hypertrophied or senescent cartilage cells found in endochondral bone formation, imbedded in a coarse-fibered matrix. A typical section is shown in Figure 2, in which part of the ground substance is seen to be calcified. Cavities surrounded by the cartilage-like tissue, and containing reticular cells and capillaries suggestive of primitive marrow, were frequently observed. Examples are shown in Figures 3, 4, and 5. Evidence of partial ossification may be discerned in Figure 3. One lesion was found in which the tissue resembled a spicule of heterotopic bone or osteoid tissue, with small cells lying in a compact, homogeneous matrix (Fig. 4). Elsewhere in the same heart another lesion was composed entirely of hypertrophied cartilage-like cells, as in Figure 2.

At their points of attachment to the heart wall, the cartilaginous structures lay beneath the endocardium in fibrous tissue continuous with the heart muscle (Fig. 5). A sheath of tissue continuous with the endocardium surrounded the remainder of the spicule that lay free in the lumen of the ventricle.

Grossly, the calcified structures were seen only in the left ventricle. Microscopic study of one heart revealed cartilage-like tissue imbedded within the wall of the left atrium (Fig. 6). The appearance of the surrounding area suggested early formation of cartilaginous tissue. Adjacent to this, the heart wall showed evidence of chronic inflammatory changes.

Calcified bodies were observed in the hearts of 7 per cent (25 rats) of the gray Norways examined, and in less than 1 per cent (7 rats) of the albinos. The discovery of some heterotopic tissue only by microscopic examination makes a more accurate estimate of its frequency impossible until a large series of preserved hearts is studied. However, on the basis of the data at hand, gray rats appear to be more susceptible to this pathologic condition, as they were to thrombosis. Moreover, the spicules in their hearts were, for the most part, decidedly larger than those in the albinos, and were found at an earlier age, the youngest gray rat being 364 days old, while the youngest albino with visible spicules was 830 days of age. The oldest gray Norway with the lesion was dissected after anesthetization at 1055 days of age; the average age for the group was 734 days. The average age of the albinos with heart spicules was 880 days. During the life of these rats no symptoms were noticed that indicated impaired efficiency of the heart.

## DISCUSSION

Calcium deposits of an amorphous appearance have been observed in the hearts of rats,<sup>2</sup> and calcification of the aortic ring, with cartilage and/or bone formation, has been reported in various animals, including rats.<sup>3</sup> In the experimental production of bone in the aortas of rabbits, Harvey<sup>4</sup> found chiefly osteoid tissue, but occasionally an intermediate tissue that resembled cartilage. This process, occurring usually in an area of calcareous degeneration, he considered to be one of metaplasia of the connective tissues. It is thought that the ossifying cartilage and bone described by us arose from fibroblasts in areas of degeneration or scarring of the heart, the cause of which is unknown. If the condition here reported is analogous to Harvey's results, it would appear that in the rats of our colony the predominant metaplastic tissue in the heart is cartilaginous in type. The fibroblasts apparently metamorphosed into large, swollen cells resembling senescent cartilage cells, frequently within a calcified matrix, but stopped short, for the most part, of transformation into tissue more nearly resembling bone.

## SUMMARY

The hearts of 336 gray Norway rats and 807 Wistar albinos were examined grossly for abnormalities.

Thrombi were found in the left atria of 24 gray Norways and 2 albinos.

Calcified structures, loosely attached to the chamber wall, were seen within the left ventricles of 25 gray rats (including 11 with thrombi in the atrium) and 7 albinos (including 1 with a thrombus).

Microscopically, the calcified structures were composed chiefly of large cells resembling the senescent cartilage cells of endochondral bone formation, although areas resembling osteoid tissue were occasionally seen. The matrix was frequently calcified, and tissue resembling primitive marrow was often present. At the points of attachment to the heart wall, the heterotopic tissue was imbedded in fibrous connective tissue.

It is assumed that the ossifying cartilage-like tissue arose from the metaplasia of fibroblasts in areas of degeneration or scarring, the cause of which is unknown.

We wish to express our appreciation to Dr. Robert C. Horn, Jr., of the Hospital of the University of Pennsylvania, for examination of the microscopic sections and advice.

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#### DESCRIPTION OF PLATES

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##### PLATE 125

FIG. 1. Heart of rat cleared with KOH and stained with alizarin-red-S. Calcified bodies are visible in the left ventricle, which was laid open.

FIG. 2. Spicule lying in the lumen of the left ventricle. Calcification and dispersal of cartilage-like cells are shown.  $\times 125$ .

FIG. 3. Marrow-like cavity within a spicule, with apparent erythropoiesis.  $\times 430$ .

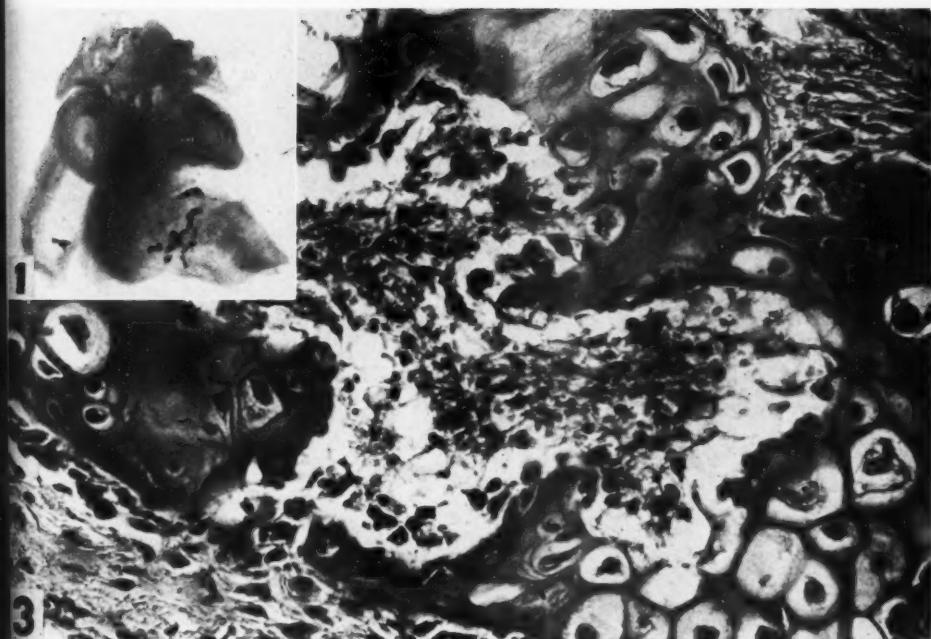
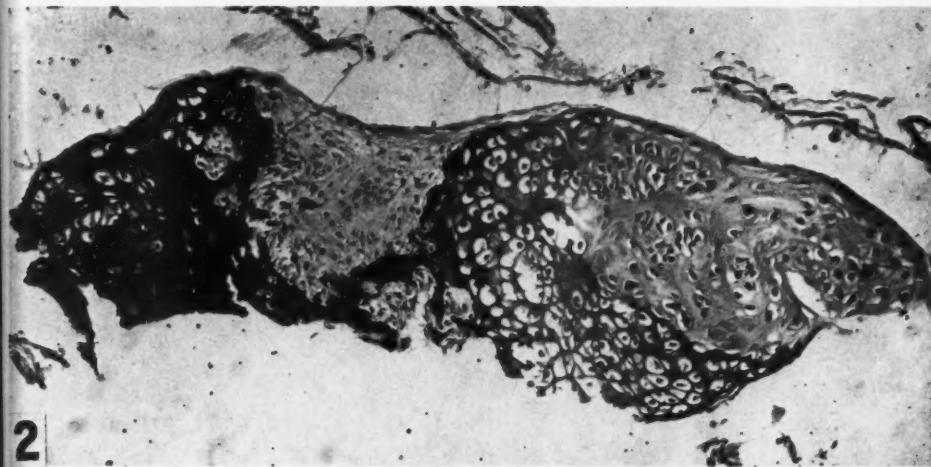


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Farris, Yeakel, and Seitner

Cartilage in the Hearts of Rats

PLATE 126

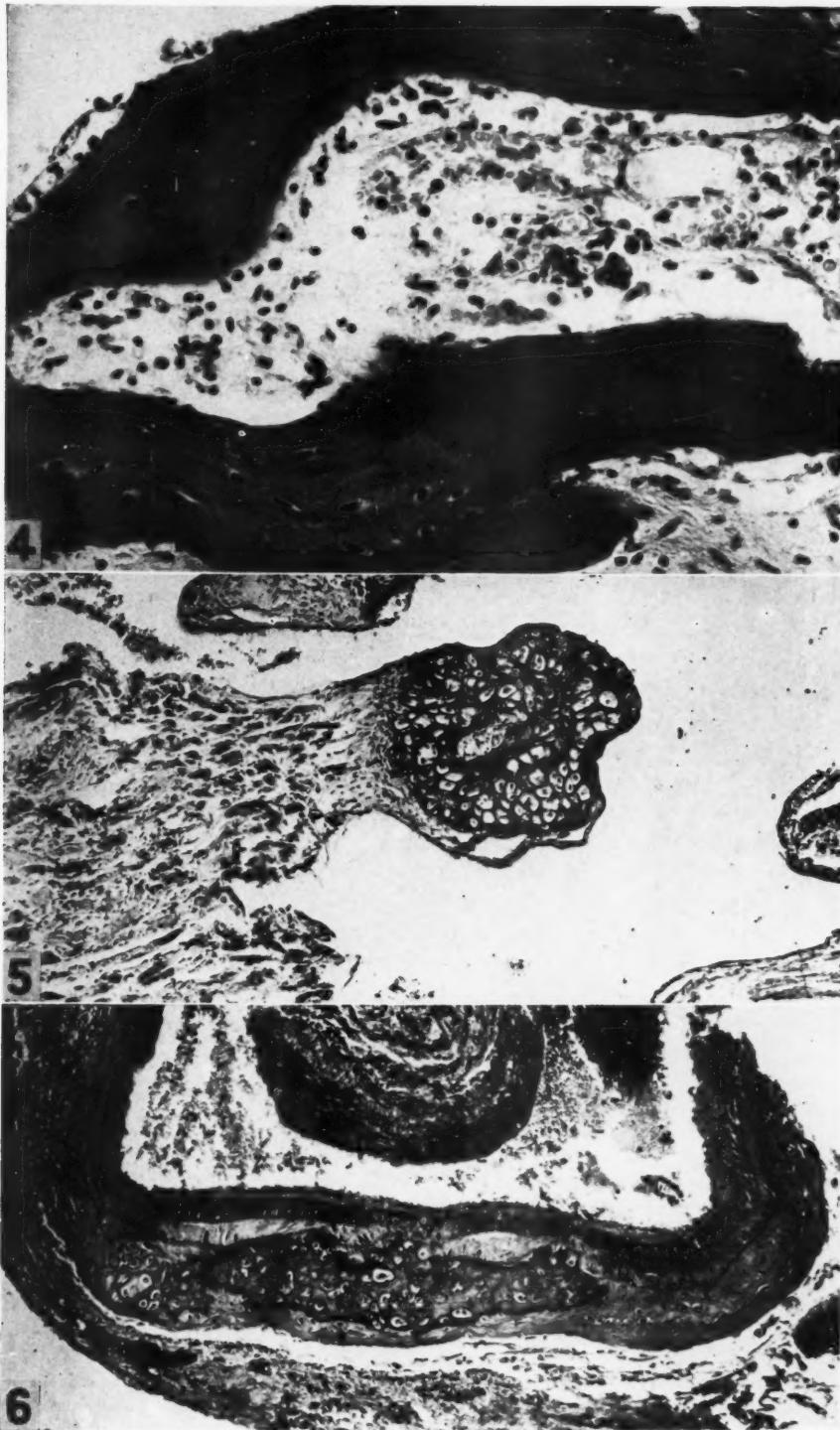
FIG. 4. Bone surrounding marrow cavity. Capillaries are visible.  $\times 395$ .

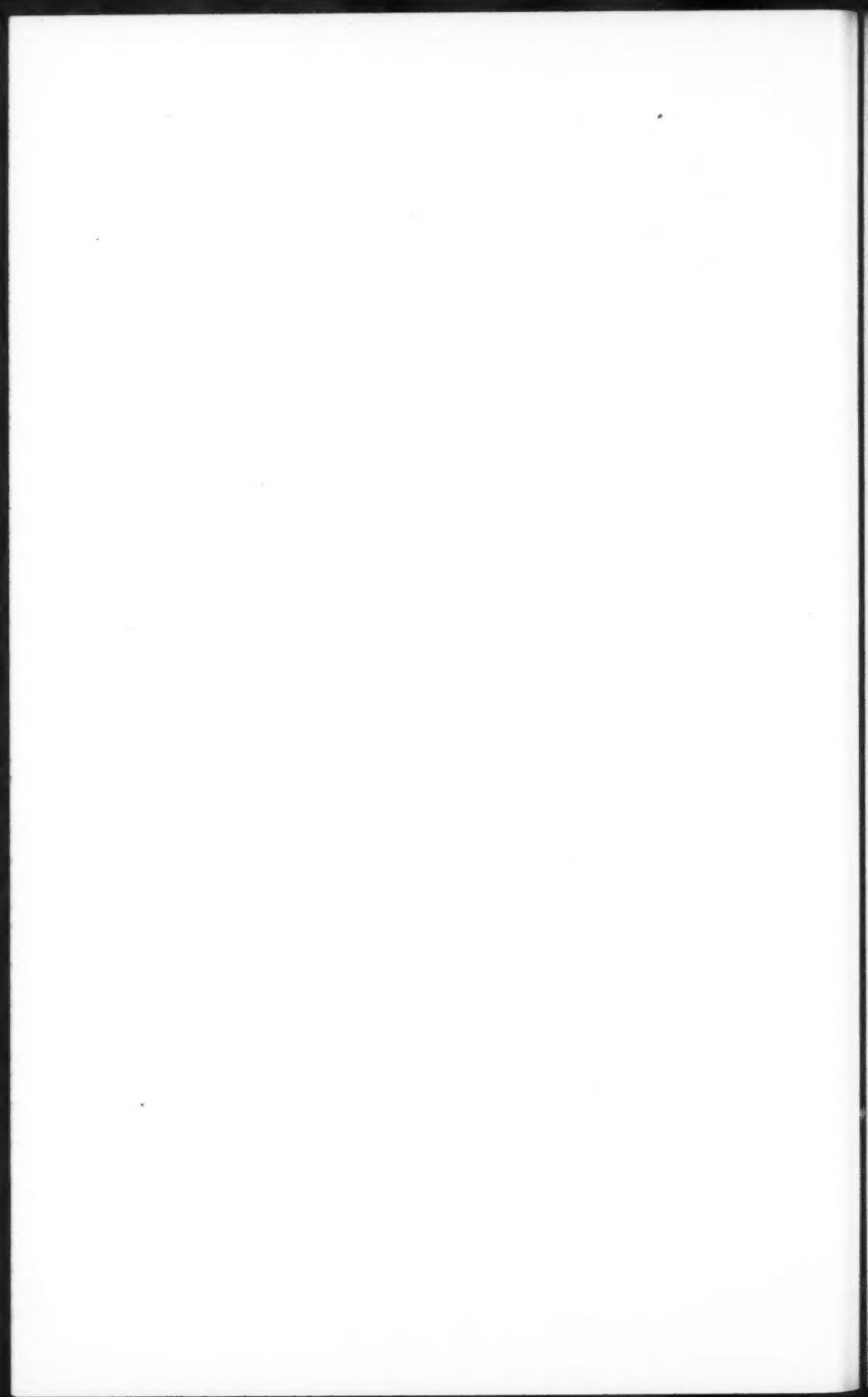
FIG. 5. Attachment of cartilaginous structure to ventricle wall.  $\times 115$ .

FIG. 6. Cartilaginous tissue imbedded in atrial wall.  $\times 115$ .









ANOMALOUS PORTAL VEIN IN MICE  
OCCASIONALLY CAUSING INTESTINAL INFARCTION \*

M. C. BOON, M.A.

(From the Department of Pathology, Cornell University Medical College,  
New York 21, N.Y.)

An anomaly of the portal vein has frequently been observed in mice of two inbred strains, and this has often proved fatal by causing hemorrhage, infarction, or obstruction of the intestine. A similar abnormality has been encountered in man.<sup>1</sup>

Normally the portal vein in mice, as in rats,<sup>2</sup> receives the splenic, superior mesenteric, and pyloric veins and then courses dorsal to the duodenum, with the hepatic artery and common bile duct, to the liver. The portal vein may, however, pass ventrally across the duodenum to the liver and remain unattached except distally where it leaves the mesentery, and proximally at its entry to the liver (Fig. 1). The hepatic artery and common bile duct are then separated from the anomalous portal vein and follow their usual path. No associated abnormalities of other blood vessels or of viscera have been observed.

In most of the observed cases this variation in the course of the portal vein had caused no signs and was only an incidental finding at autopsy. In a number of instances, however, loops of the small intestine had herniated through the space behind the vein, causing obstruction by elongating and compressing the vein, with resulting hemorrhagic infarction of the mesentery, intestine, and spleen (Figs. 2 and 3). In a few cases, the vein appeared to have strangulated the intestine, causing distention of the intestine and stomach above the point of constriction. Perforation and peritonitis were not seen.

This abnormality has thus far been observed at autopsy in more than 380 mice, in 24 of which intestinal infarction or obstruction, as described above, caused death. The malposition occurred most frequently in mice of the Ak stock, a high-leukemia stock of mice which has been inbred in this laboratory by brother-sister matings for more than 30 generations. The total incidence of the anomaly in the Ak stock has been 26 per cent. Table I shows that in three sublines of the Ak stock the frequency varied, but that in each subline it occurred approximately twice as often in female as in male mice. These figures are undoubtedly lower than the actual numbers since the condition may be difficult to recognize at autopsy, especially if post-mor-

\* These observations were made in the course of studies on leukemia, carried out with support from The Lady Tata Memorial Trust, The Jane Coffin Childs Memorial Fund for Medical Research, and The International Cancer Research Foundation.

Received for publication, May 17, 1945.

tem decomposition has become advanced or if the vein is empty and appears as a mere fibrous strand between the liver and the mesentery. Two per cent of mice of the Rf stock, which is a highly inbred, low-leukemia stock, have also been found to have this abnormality. It has appeared in various hybrid combinations of the Ak mice with other

TABLE I  
*Incidence of Anomalous Position of Portal Vein in Ak and Rf Inbred Strains of Mice*

Stock of mice	Females			Males		
	Number observed	With anomaly		Number observed	With anomaly	
		Number	Per cent		Number	Per cent
Subline Akl	137	61	45	90	22	24
Subline Akn	161	53	33	113	15	13
Subline Ako	339	93	27	248	34	14
Total Ak	637	207	32	451	71	16
Total Rf	130	4	3	78	0	0

stocks; the incidence appears to be lower in these than in the Ak mice, but the data are insufficient for analysis.

In an attempt to determine a genetic basis for this congenital variation, two litters of Ak mice in which the course of the portal vein had been ascertained by laparotomy were bred in various combinations, as shown in Table II. Of the 7 mice in each of these litters, 2 in one and

TABLE II  
*Incidence of Anomalous Position of Portal Vein in Offspring from Selected Ak Mice in Which the Course of the Portal Vein Was Known*

Parents	Offspring					
	Females		Males		Total	
	Produced	With anomaly	Produced	With anomaly	Produced	With anomaly
♀ normal × ♂ normal	6	3	6	1	12	4
♀ normal × ♂ with anomaly	8	4	14	3	22	7
♀ with anomaly × ♂ normal	15	8	9	2	24	10
♀ with anomaly × ♂ with anomaly	5	1	16	5	21	6
Total	34	16	45	11	79	27

5 in the other had the abnormality. Offspring with and without the anomaly were produced by each of the mating combinations, with no significant differences in the proportion obtained from the various matings; and the total incidence in offspring from all of these matings agreed closely with that obtained by unselected breeding of the Ak stock. Despite the slightly different percentages of mice with this venous malposition in the various sublines of the Ak stock, no tend-

ency was observed for families within each line to have a high or a low incidence of this condition.

Thus a genetic basis for this anomaly has not been definitely established. The absence of significant differences among the progeny from various mating combinations is similar to findings on the incidence of leukemia in various mating combinations of the Ak stock. Spontaneous leukemia has been observed in approximately 77 per cent of the females and in 61 per cent of the males of the Ak mice. The figures vary slightly among sublines of the stock; however, in each subline the incidence in the progeny is the same, whether both, one, or neither of the parents develop leukemia.<sup>3</sup> It is noteworthy that both leukemia and this anomaly occur more frequently in female mice than in males of the Ak stock, and both are infrequent in mice of the Rf stock. There has been, however, no correlation between the incidence of leukemia and that of this venous abnormality. In the case of leukemia, these findings are interpreted by assuming that the incidence of leukemia as determined by hereditary factors is to some extent controlled by extrinsic factors.<sup>4</sup> Such an explanation does not seem adequate for a congenital anomaly.

Anomalies of the portal vein in man are frequent in association with partial or complete situs inversus of the abdominal viscera, though rare in its absence.<sup>1</sup> Pernkopf<sup>1</sup> has reported a case in man in which the portal vein was in the preduodenal position but was not accompanied by situs inversus, and has discussed numerous variations in the embryological development of the portal vein. This anomaly results from variations in the development of the portal vein from the peri-intestinal rings formed by the vitelline veins in the embryo. Anomalies of the intra-embryonic portion of the vitelline vein in a pig embryo and a human embryo have actually been noted by Begg.<sup>5</sup>

The observation<sup>6</sup> that various congenital defects in infants are produced by infection of the mother with rubella during the early months of pregnancy suggests the possibility that instead of, or in addition to, a genetic factor, some mild or latent infection might be responsible for the occurrence of the anomalous course of the portal vein in mice.

#### SUMMARY

An anomalous preduodenal position of the portal vein, not associated with other vascular anomalies or variations in the position of the abdominal viscera, has been observed in 380 mice. In 24 mice it caused death by producing intestinal obstruction or infarction. A similar anomaly has previously been described in man.

Most of the cases occurred in mice of the highly inbred stock Ak,

in which the incidence of the anomaly was 26 per cent. It has also been observed in 2 per cent of mice of another highly inbred stock (Rf) and in hybrids of the Ak stock. The incidence of the anomaly in female mice was approximately twice that in males.

Attempts to determine a genetic basis for this venous malposition by breeding of selected Ak mice were unsuccessful.

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#### DESCRIPTION OF PLATE

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##### PLATE 127

FIG. 1. A mouse in which the portal vein is ventral to the duodenum. The liver has been turned back to expose the vein and duodenum.

FIGS. 2 and 3. Intestinal infarction in mice, produced by herniation of loops of intestine behind the portal vein in the anomalous position. In Figure 3, the vein is the thin strand indicated by the arrow.





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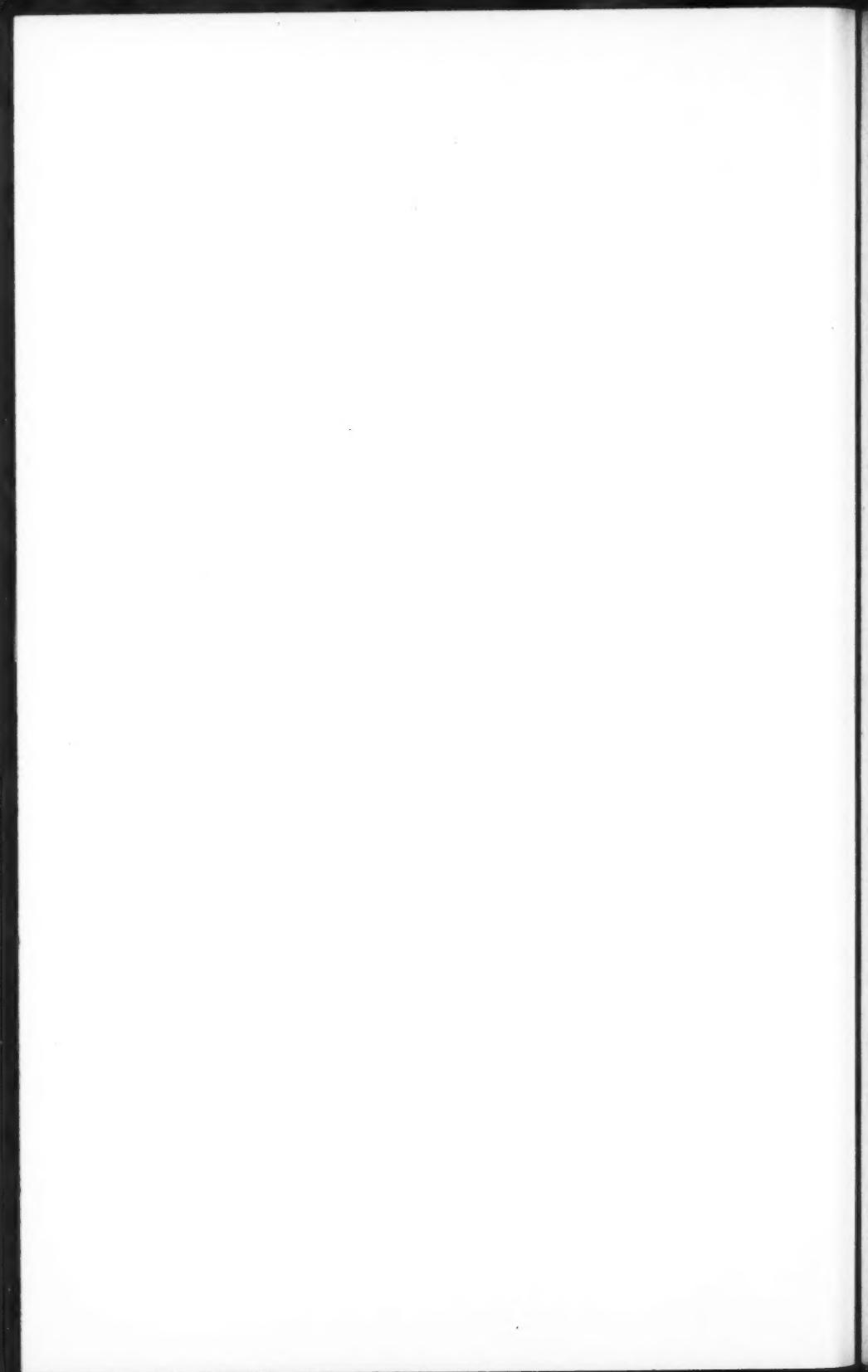


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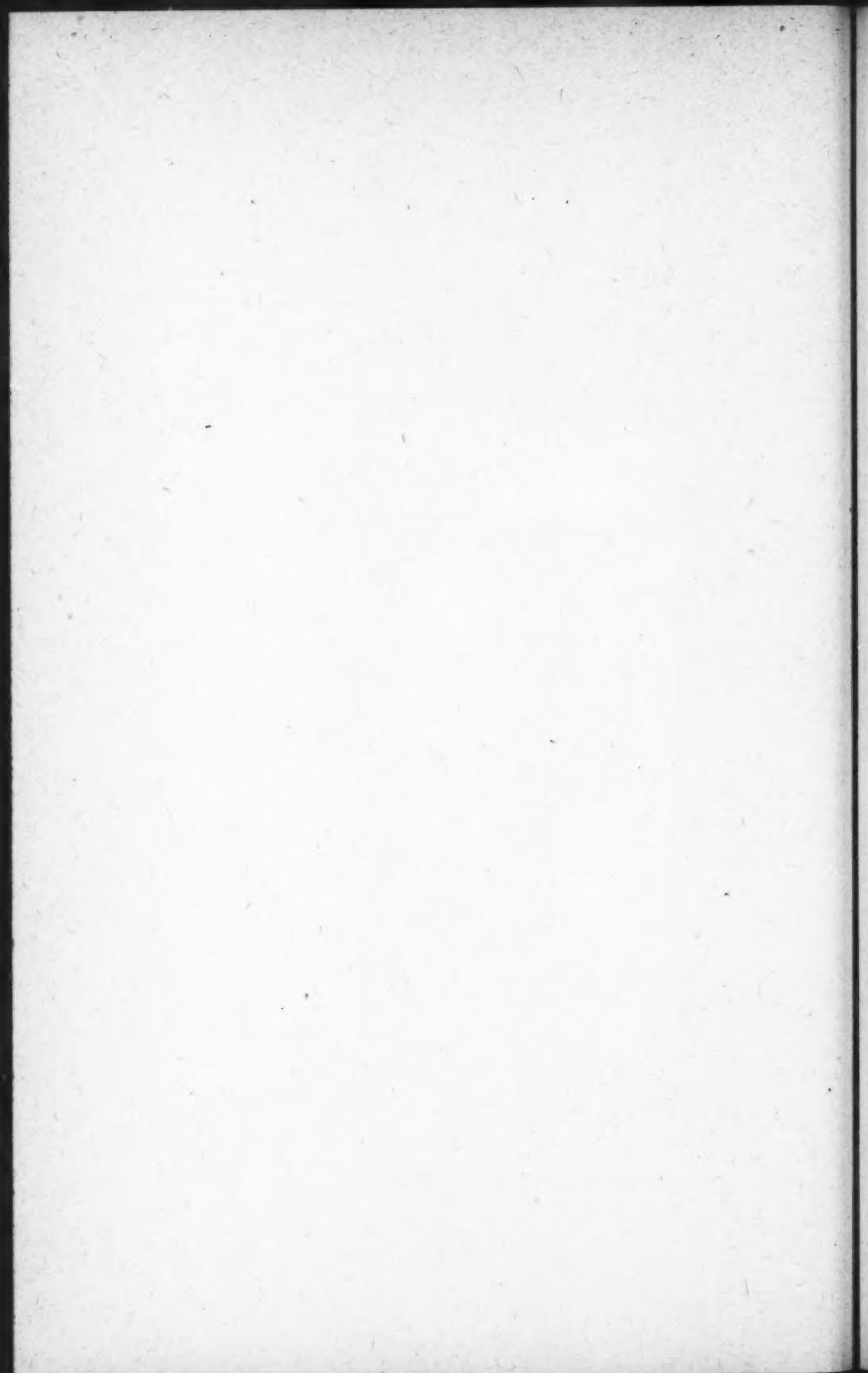
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FORTY-THIRD ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS

CHICAGO  
MARCH EIGHTH AND NINTH, 1946



THE AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS

Forty-Third Annual Meeting, University  
of Chicago, Chicago, Illinois  
March Eighth and Ninth, 1946

PRESIDENT CANNON IN THE CHAIR

BUSINESS MEETING  
March Eighth, 1946

Upon nomination of the Council, the Association voted to elect the following officers:

<i>President</i>	WILEY D. FORBES
<i>Vice-President</i>	MALCOLM H. SOULE
<i>Secretary</i>	HOWARD T. KARSNER
<i>Treasurer</i>	ALAN R. MORITZ
<i>Incoming Member of Council</i>	TRACY B. MALLORY
<i>Assistant Secretary</i>	WILLIAM B. WARTMAN

The Secretary announced the election of Dr. Malcolm H. Soule to succeed himself as Associate Editor of *The American Journal of Pathology* for the ensuing year.

The Council announced the election of Dr. Howard T. Karsner to succeed himself on the Editorial Board of *The American Journal of Pathology*.

The Secretary announced the election of the following new members:

Margaret Bevans, New York	Elson B. Helwig, St. Louis
Richard F. Birge, Des Moines	George K. Higgins, New York
Charles M. Blumenfeld, Sacramento	Leslie S. Jolliffe, Newton Highlands, Mass.
Milton D. Bosse, Pittsburgh	John F. Kent, Washington
Henry Bunting, New Haven	Philip M. LeCompte, Brookline, Mass.
Arthur A. Case, Columbus, Ohio	Aaron E. Margulis, New York
Robert C. Dunn, Bethesda, Md.	Paul Michael, Oakland, Calif.
Stanley H. Durlacher, New Haven	Nathan Mitchell, Albany
Curtis M. Flory, New York	Richard H. Follis, Durham, N.C.
Richard H. Follis, Durham, N.C.	Robert H. More, Montreal

Leo D. Moss, Olean, N. Y.  
Francis P. Parker, Atlanta  
S. B. Pessin, Milwaukee  
William R. Platt, St. Louis  
John T. Read, Columbus, Ohio  
Stanley L. Robbins, Brookline,  
Mass.  
Philip Rosenblatt, New York  
Vinton D. Sneeden, Portland, Ore.  
Joseph Stasney, Philadelphia  
James S. Taylor, Kingston, N.Y.  
Wilbur C. Thomas, Winston-  
Salem, N.C.  
Paul A. Van Pernis, Chicago  
Ohio O. Williams, Phoenix, Ariz.

The Secretary reported the reinstatement to membership of Drs. Frederic H. Foucar, Joseph C. Ehrlich, and Arthur J. Vorwald.

The Council voted to accept with regret the resignations of Drs. Stuart Graves, Calvin G. Page, and William H. Woglom.

The Council voted to record with regret the deaths of Drs. Sam S. Blackman, Jr., Newton Evans, John C. Grill, Edgar S. Ingraham, Jr., Lewis H. Koplik, and Isaac Levine.

The Secretary announced that the next meeting of the Association will be held as guests of the Jefferson Medical College, Philadelphia, Pennsylvania, on the Friday and Saturday immediately preceding the meetings of The Federation of American Societies for Experimental Biology.

The Secretary announced that the Council had voted to have a symposium in 1947 on "Necrotizing Hepatic Injury and Sequels" and appointed Lt. Col. Balduin Lucké as referee.

## SCIENTIFIC PROCEEDINGS

THE BETA GRANULES IN THE ISLETS OF LANGERHANS IN DIABETES MELLITUS.  
E. T. Bell, Minneapolis, Minn.

*Abstract.* With the use of Gomori's stain the beta granules of the islets of Langerhans have been studied in 50 pancreases from nondiabetic and 30 pancreases from diabetic patients. The beta granules were clearly shown in all of the non-diabetic persons.

Of the 30 patients with diabetes, the beta granules were completely absent in 11, and present in very small quantity in 10 others. In these 21 cases the diagnosis of diabetes was made with the Gomori stain, and, since only 9 of them showed hyaline islets, there were 12 cases which could be recognized only by this stain. In 5 cases the beta granules were only moderately reduced, so that the diagnosis of diabetes was uncertain; and in 4 cases the beta granules were present in normal numbers. Two of the cases with normal granulation were clinical examples of severe diabetes. The beta granules are evidently related in some way to the formation of insulin, but their significance is not understood.

One islet cell adenoma of the pancreas, associated with severe hypoglycemia, showed only a few beta granules.

### *Discussion*

(Dr. Israel Davidsohn, Chicago, Ill.) What about the time interval since death? Did that affect the stain?

(Dr. Bell) Post-mortem changes did not affect the islets any more than they did the acinar cells of the pancreas. Material obtained 12 hours after death shows them quite well, and in any pancreas that was not autolyzed you could see the beta granules, so we did not have to have a fresh pancreas to make this stain work.

LYMPHADENOID GOITER. ITS DIFFERENTIATION FROM CHRONIC THYROIDITIS. C. C. Parmley (by invitation) and C. A. Hellwig, Wichita, Kan.

*Abstract.* The histologic findings in 14 lymphadenoid goiters are presented because alterations of the thyroid epithelium are, in our opinion, more important characteristics of this disease than the accumulations of lymphoid tissue. These epithelial changes have not been correctly interpreted in recent studies.

Two types of epithelial alteration are present in all of our cases: (1) small slit-like acini with high cuboidal epithelium and loss of colloid, and (2) strands of large, pale oxyphilic cells which resemble liver or adrenal cells. The latter are identical with the so-called Hürthle cells. Hürthle cell tumors occur almost exclusively in women over 40 years of age, the same as lymphadenoid goiters. These large, pale cells represent, in our opinion, an attempt to compensate for the exhaustion of colloid in the small acini.

Lymphadenoid goiter is explained best by a sequence of events similar to those of thiouracil goiter, except that the disturbance of the cyclic activity of the thyroid acinus is not initiated by a chemical agent but by the decline in ovarian function. The resulting excess of thyrotropic hormone will, as in thiouracil goiter, produce loss of colloid and hyperplasia of the thyroid epithelium associated with hypo-function.

### *Discussion*

(Dr. Carl V. Weller, Ann Arbor, Mich.) I would like to ask the speaker how many lymphocytes he requires in the thyroid before he designates a particular example as belonging to the group of lymphadenoid goiters.

(Dr. William Boyd, Toronto, Ont.) I regret that the speaker does not stick to the title of the paper on the program, which is the differentiation of lymphadenoid goiter from chronic thyroiditis. One of the most difficult problems we have to face is to determine the relationship between these two conditions. I hope before closing Dr. Parmley will refer to that. Does he consider these conditions entirely independent, or may one after a period of years merge into the other?

(Dr. William H. Harris, New Orleans, La.) Were follicles or germinal centers present? I ask this, as it indicates local production. The intimate relationship of lymphoid and thyroid structures in embryonal development must also be considered. The lateral aberrant lympho-thyroid tumors are evidences of gross exaggeration.

(Dr. Hamilton Fishback, Chicago, Ill.) I would like to know if any of the patients had had previous iodine therapy.

(Dr. Wiley D. Forbus, Durham, N.C.) May I ask if in your experience there has occurred any appreciable increase in the incidence of this condition since the introduction of the sulfa drugs in therapy?

(Dr. Parmley) As regards Dr. Weller's question: the amount of lymphoid tissue necessary for this type of goiter we have found to vary to some degree, but on the average as previously stated, these glands consist of approximately one-third lymphoid tissue.

As Dr. Boyd pointed out, time did not permit us to discuss the question of differentiating lymphadenoid goiter from chronic inflammation. We intend to do this in the paper to be published. We consider the essential difference between chronic thyroiditis and lymphadenoid goiter to be the presence in the latter of large, pale eosinophilic cells, the absence of tubercle-like giant cells, and of fibrosis throughout the gland. Also, this type of goiter occurs in the female around the menopausal age. Chronic thyroiditis occurs as a rule in younger persons, with equal frequency in both sexes. We consider these conditions entirely independent and one after a period of years will not merge into the other.

We did find germinal centers quite frequently throughout our specimens. They varied in number and size and were present in all but two or three glands.

In answer to Dr. Forbus' question, I cannot speak too definitely about the frequency of this type of goiter since the advent of the sulfa drugs. In the last few years we have noticed a definite increase in this type of goiter, namely, among 500 goiters there were 14 lymphadenoid goiters, *i.e.*, 2.8 per cent.

In answer to Dr. Fishback's question, all of these patients with lymphadenoid goiter had iodine preoperatively. One patient was on Lugol's solution 6 months before operation.

#### THE PATHOGENESIS OF TUBEROUS BONE FORMATION IN THE LUNGS. Kornel L. Terplan, Buffalo, N. Y.

*Abstract.* In the course of systematic search for calcified tubercles small bony particles frequently can be encountered in the lungs, either in very small numbers (from one to four) or in larger numbers symmetrically distributed. As single structures these are known in the literature as "osteomas," the disseminated type as the tuberous form of bone formation. Such bony structures were seen in larger numbers in cases of cardiac insufficiency by Freeman-Dahl and in patients with mitral stenosis by Gross and Janker, who studied their roentgenologic appearance and increase in size over a period of from 4 to 8 years. Wells reported one such case of disseminated bone formation in mitral stenosis. The pathogenesis of this bone formation was admittedly not clear. The fibrosis of the lungs was found by Wells to be different from that in ordinary chronic passive congestion. The connective tissue increase was more focal, hemosiderin pigment deposition was slight. He thought that a combination of chronic interstitial pneumonia with chronic pul-

monary congestion might be instrumental in bringing about this tuberous ossification.

A case of mitral stenosis in a young adult with a very large number of these small, tuberous, bony structures offered a good opportunity to study their pathogenesis. In the parts of the lungs in which there was no roentgenologic evidence of bone formation, histologic pictures were seen which seemed to represent the early phases of, and the actual matrix for, this bone formation. Tuberous bone formation is, on the basis of our morphologic analysis, the result of thrombosis in the septal capillaries. Capillary thrombi were found either *in situ* within the septa, or, more often, protruding in various forms into the alveolar space, with one wall still adherent to the septum, or extending in polypous fashion into the lumen, or, within the alveolar spaces. It is easy to demonstrate morphologically the capillary thrombotic nature of these structures. Blood corpuscles and blood fluid remain finally, apparently free or suspended by a few thin threads of mesenchymal cells within the capillary membrane; in various states of hyalinization of the thrombus, mesenchymal (septal) cells can be seen attached to the surface of the detached thrombosed capillary. These mesenchymal cells are apparently concerned with the gradual formation of bone. No bone formation was seen in these capillary thrombi, in contradistinction to the usual "phlebolith" as found in the pulmonary arteries. It is suggested that such capillary thrombi in certain phases of their formation, especially when in but loose connection with the septa, might easily be forced into other parts of the lung by increased expiratory pressure, especially by coughing. They might be found in the sputum of patients in whom this tuberous bone formation can be demonstrated radiologically.

#### CRYSTALLINE ESTER CHOLESTEROL AN IRRITANT. Timothy Leary, Boston, Mass.

**Abstract.** In the embedding of tissue in paraffin or celloidin, fatty substances, including cholesterol, are extracted from the tissues. Fresh tissue or recently fixed formalin tissue is essential for the study of crystalline cholesterol, which is visible as such only under polarized light.

Gye and Purdy (*Brit. J. Exper. Path.*, 1922, 3, 86-94; 1924, 5, 238-250) injected silica sol (colloidal silica) intravenously in rabbits and produced, under low dosage over a considerable period, cirrhosis of the liver, enlargement of the spleen, and chronic nephritis, which lesions they looked upon as characteristic of chronic silica poisoning. Rabbits fed cholesterol over long periods will develop, in addition to atherosclerosis, cirrhosis of the liver, enlargement of the spleen, and chronic nephritis. These lesions arise following the precipitation in the liver of cholesterol ester crystals. The crystals are treated as foreign bodies. They are taken over from the liver cells by Kupffer cells, are carried into the lymph and blood streams by these macrophages, and stimulate the growth of connective tissue wherever they are stored within the lipophages. The most efficient form of silica as an irritant is in crystals 1 to 3  $\mu$  in diameter (Gardner, L. U., and Cummings, D. E. *Am. J. Path.*, 1933, 9, 751-763). Cholesterol ester crystals are taken into lipophages in particles (droplets) 1 to 3  $\mu$  in diameter. Both silica and ester crystals are treated as foreign bodies, provoke phagocytosis, and stimulate the growth of fibrous tissue.

#### CRYSTALLINE ESTER CHOLESTEROL AND ATHEROSCLEROSIS. Timothy Leary, Boston, Mass.

**Abstract.** Evidence is presented that crystalline ester cholesterol is present in all active lesions of atherosclerosis. Solid crystals of cholesterol may arise in late lesions through the necrosis of lipophages carrying ester crystals and the splitting of the esters. Only advanced calcified or repaired lesions are free from crystalline cholesterol. A progressive sequence of changes in the evolution of (a) aortic and

(b) coronary sclerosis is shown. Atherosclerosis cannot be produced experimentally by wear and tear methods. Crystalline cholesterol appears to be the essential substance in human and experimental sclerosis.

#### Discussion

(Dr. E. T. Bell, Minneapolis, Minn.) I would like to ask Dr. Leary if it is not true in human atherosclerosis that you can find occasionally small early lesions that are pale whitish, and in which, on microscopic examination, you get the impression that the edema and loosening of the tissues precede the deposit of the cholesterol. Most of us have had the idea that the cholesterol deposit was secondary to some injury of the aorta, and Dr. Leary apparently thinks that the cholesterol is deposited first and that it is responsible for the atheroma.

(Dr. Leary) The idea that cholesterol deposit in atherosclerosis is secondary to some injury of the aorta is based on the Virchow-Aschoff "imbibition theory" which has delayed recognition of the real cause of the disease. Klotz first, and Anitschkow later, insisted that the aortic intima showed no evidence of injury in the earliest lesions of the disease, which were marked by the appearance of cholesterol within macrophages in the subendothelial layer of the intima. They did not know the sources of the macrophages, which are Kupffer cells from the liver as I have shown. Earliest lesions appear as atheromatous processes in the aorta of youth and also in the ascending aorta even in age. With adequate human material it is possible to collect series of ascending aortae with crops of pinhead lesions that are colored orange-yellow in the earliest stages, paler yellow in mid-course, and gray-yellow to gray-white in later stages. These stages represent the waxing and waning of atheromatous lesions in which the cholesterol esters, after being deposited, are removed by a mechanism corresponding to that which removes the excess cholesterol from atheromatous lesions in youth. It is this mechanism which protects the ascending aorta from advanced lesions. When this mechanism is destroyed, as by syphilitic aortitis, continuous atherosclerotic lesions appear in the ascending aorta. The earliest lesions are made up of macrophages filled with cholesterol ester crystals beneath the aortic endothelium. Pale whitish lesions with edema are advanced and not the earliest lesions.

(Dr. A. R. Crane, Norfolk, Va.) I would like to ask if Dr. Leary has noticed the occurrence of hemorrhages in these atheromatous lesions with any degree of frequency, and has he had an opportunity to note any changes in the prothrombin time of these animals.

(Dr. Leary) First, in reference to hemorrhages, they are always a late phenomenon in atherosclerosis; in the coronaries we get vascularization from the coronary lumen in late lesions, and one of the means of producing thrombosis without question is vascular spasm which ruptures the capillary network and leads to hemorrhage and to fibrinoid necrosis, extending to the endothelium and causing thrombosis. Hemorrhages are a very late phenomenon under any circumstances. With reference to the prothrombin time, I have done no work on that.

(Dr. William H. Harris, New Orleans, La.) I would like to ask what Dr. Leary considers the pathogenesis in those lesions with manifest mechanical or physical relationships, such as those at the orifices of the intercostals, or as shown in industrial employment as "one leg" hyperactivity and the like. How does he link such with crystalline ester cholesterol irritation?

(Dr. Leary) The coronary arteries, notably the epicardial branches, are the most common and most important sites of atherosclerotic lesions. During systole the epicardial branches of the coronaries are distended with blood under systolic pressure, while the muscular branches are compressed by the contracting ventricles. There is a definite pallor of the contracting ventricles, notably the left. This results in a slowing of the blood in the epicardial branches, sometimes almost to stasis, so

that there is opportunity for the sticky macrophages to adhere to the wall. Similarly, at the end of the systole temporary stasis favors invasion of the intima in the ascending aorta. About the orifices of the intercostal and other branches of the aorta there is a break in the continuity of the forward movement of the blood. The swirling currents about the orifices presumably produce conditions favorable to the adhesion of the cells to the wall. In the experimental rabbit the origin of the lipophagic cells in the liver sinusoids, their phagocytosis of crystalline esters, their escape into the blood stream, their passage through the lungs, and their entrance into the aortic intima can be followed in detail. The irritant quality of cholesterol ester crystals is responsible for the progression of the disease.

**TUMORS OF THE TESTIS.** Nathan B. Friedman, Capt., M.C., Washington, D. C.

*Abstract.* A simplified classification of tumors of the testis has been set up, based on study of more than 800 such neoplasms collected at the Army Institute of Pathology between 1941 and 1946. Ninety-nine per cent of them fall into one of the following categories: seminoma, embryonal carcinoma, chorioepithelioma, teratoma, teratocarcinoma, or interstitial cell tumor. Serial sections of small tumors have revealed that not all monocellular neoplasms of the testis originate from teratomas. Seminoma and the embryonal carcinoma differ not only in fundamental cell type but in biologic behavior and prognosis. Consequently, the misleading term "embryonal carcinoma with lymphoid stroma," which does not designate a pathologic entity, should be abandoned. The chorioepithelioma should be considered a subvariety of embryonal carcinoma because of the tendency toward trophoblastic differentiation evident in many carcinomas and the frequent occurrence of mixed, intermediate, and transitional forms.

A new term, "teratocarcinoma," is proposed for the large group of pleomorphic tumors in which both differentiated teratoid structures and malignant elements are present. The view that the carcinomatous component of such mixed neoplasms does not necessarily arise from the teratomatous portion is supported by the fact that it is usually an embryonal carcinoma and is almost never of a specialized epithelial type. Moreover, teratoid differentiation in embryonal carcinomas does take place. Virtually all metastases of embryonal carcinomas develop as monocellular embryonal carcinomas or chorioepitheliomas. Roughly half of the teratocarcinomas which metastasize give rise to tumors with teratocarcinomatous structures and half to pure embryonal carcinomas. Choriomatous characteristics may be evident in the metastases of an embryonal carcinoma or teratocarcinoma even when they are not manifest in the primary. The immediate prognosis for embryonal carcinoma and chorioepithelioma is very bad. Since it is almost as serious for teratoma, this neoplasm should be termed "adult" and not "benign." Mixed teratocarcinoma metastasizes less readily and has a lower early mortality than either carcinoma or teratoma. The immediate prognosis for seminoma, by comparison with the other tumors, is very good.

The element from which testicular tumors other than seminoma arise possesses the developmental potencies of normal germ cells, for it can differentiate into both somatic and trophoblastic tissues. It has been suggested that the seminoma represents an attempt to reproduce testicular tissue itself, a theory which is attractive but not established.

*Discussion*

(Dr. Nicholas M. Alter, Jersey City, N. J.) I would like to ask one question in regard to nomenclature which is rather academic. Since the ovarian dysgerminoma is identical with the dysgerminoma of the testis, why not call it dysgerminoma of the testis? It does not deserve the name "seminoma" because it does not derive from seminiferous tubules. Perhaps the time will come when these tumors can be classified into immature and mature teratomata.

(Dr. Friedman) The only reason I retained the word "seminoma" is because so many people use it, and I agree with Dr. Alter that this tumor is an identical tumor with the ovarian dysgerminoma. The term, "embryonal carcinoma with lymphoid stroma," has not been used; this is usually a seminoma, but some of the embryonal carcinomas have a lymphoid stroma. In mortality statistics the difference is amazing; only 1 per cent of patients with seminomas have died thus far, whereas 25 per cent of those with embryonal carcinomas have succumbed. I think if I can ever find out what the seminoma is, I will have a name for it; the name will suggest itself. I do not want to change it because I have nothing better to call it. The term, dysgerminoma, is equally acceptable, but I do not think they should all be called teratomas, and I am not sure where the seminoma cells and the seminoma tumor fit into the scheme.

(Dr. Alfred Plaut, New York, N. Y.) How often has a giant cell reaction in the necrotic portion of the lymphoid stroma been found, as is so frequently found in the corresponding ovarian tumor; and how often have isolated areas of cartilage been found in a tumor which looks like a seminoma?

(Dr. Friedman) I cannot give you the percentage incidence of granulomas in seminomas, but I would guess it is somewhere in the neighborhood of 15 or 20 per cent. As a matter of fact, we have a few tumors in which there was no seminoma tissue left except for an occasional isolated seminoma cell, and the whole mass was granulomatous. That is another reason for abandoning the lymphoid stroma idea, because if we would follow that we would have to say "with eosinophilic stroma," or "with epithelioid stroma," or "with granulomatous stroma," etc., as the case might be. I do not think the granulomas are invariably associated with foci of necrosis.

With regard to the finding of isolated lumps of cartilage, we have made serial sections when all of the tumor was submitted and it was small enough to section serially. Those which were too large were submitted to multiple block study. In a number of seminomas teratoid foci have appeared. In that simplified pie-shaped diagram which I showed, the teratomas associated with seminomas were combined under teratoma. That is an over-simplification for purposes of illustration, and seminoma does occur in combination with the other tumors. Seminomas in which there is a small focus of cartilage and usually some glands, may be side by side with teratomas as distinct entities. The significance of this is not clear, but such tumors would fit into the group "teratoma with seminoma."

(Dr. Joseph Tannenberg, Batavia, N. Y.) I would like to ask if Dr. Friedman has any information as to the life-expectancy of the patients after surgical removal and eventual x-ray treatment of the several types of tumors of the testis mentioned in his report.

(Dr. Friedman) We are a little restricted at the present time because the follow-up through the Veterans' Administration has not been worked out yet, so that our follow-up is only the immediate follow-up. There will be a long-range follow-up through the Registries. I have only the immediate follow-up now, 6 to 12 months at Army Hospitals. After the patient has his tumor removed and gets well, either he goes home or to the Veterans' Administration. All I can tell is what happened in Army hospitals, and it may be that the next year or 5 years will change the picture. The immediate expectancy for seminoma is very good. Only 1 per cent of our 300 patients with seminomas are dead. Twenty-five per cent of the patients with embryonal carcinomas and chorioepitheliomas are dead. The surprising thing is that of those with adult teratomas almost 20 per cent are dead. The deaths are mostly from the wicked metastasizers differentiating in the direction of chorioepithelioma. What the others are going to do I cannot say, but there is a sharp dichotomy between the teratoid group and the seminoma group. When Dr. Moore came on as civilian consultant at the Institute one of the first things he

asked was "can the seminoma be a benign tumor?" It is not a benign tumor, but the disparity is so striking that it points out these differences.

**ETIOLOGIC FACTORS IN PATIENTS WITH CARCINOMA OF THE PENIS AND IN CONTROL GROUPS.** Robert Schrek, Maj., M.C., and (by invitation) Herman Lenowitz, Hines, Ill.

*Abstract.* The clinical group under investigation consisted of 139 men with carcinoma of the penis. The data on the control groups were obtained from the records of patients with cancer and from interviews with men in the hospital. The percentage of colored patients was 28.1 per cent for the men with penile cancer and 6.4 to 7.4 per cent for the control groups. None of the men with cancer of the penis were circumcised as babies, but 12.8 per cent of the white and 17.9 per cent of the colored control patients were circumcised early in life. Circumcisions during boyhood and manhood were as frequent in the clinical as in the control groups. Syphilis occurred four times and gonorrhea two times as frequently in men with penile lesions as in the control groups. The incidence of both venereal diseases was twice as high in colored as in white patients. Circumcised and noncircumcised men had the same incidence of venereal disease. It is estimated that colored and white men free of venereal disease had equally low incidence rates of carcinoma of the penis. Colored men with syphilis or gonorrhea had a much higher incidence of penile cancer than white men with venereal disease. The high frequency of carcinoma of the penis in colored men could not be attributed to differences in incidence of circumcision or venereal disease or to racial immunity, but may possibly be due to differences in sex hygiene.

*Discussion*

(Dr. Alfred Plaut, New York, N. Y.) There is a curious similarity in the occurrence of carcinoma of the penis and carcinoma of the uterine cervix. As most of you know, carcinoma of the uterine cervix is rarer in Jewish women than in other populations. This applies to numerous countries; the statistics are available for the United States, for Austria, Germany, and England. It would be extremely interesting if statistical studies such as that by Dr. Schrek could be carried out in a parallel way for carcinoma of the uterine cervix.

(Dr. Joseph Tannenberg, Batavia, N. Y.) I should like to underline the warning of Dr. Plaut; there are so many other factors which are more important in the pathogenesis of penile carcinoma than this obvious mechanical factor of circumcision. In addition to the type of tumor mentioned by Dr. Plaut, I would like to mention carcinomas of the gallbladder, bile duct system, and pancreas, the incidence of which is quite different in different races or strains of the white population. Here in this country, particularly in the State of New York, I have found during the last decade that carcinoma of the gallbladder and the bile duct system is a rather rare disease, much less frequent than carcinoma of the pancreas. In Germany, however, where I saw about 2000 autopsies annually over a period of 12 years, carcinoma of the gallbladder and of the common bile duct is a very common disease, at least ten times as frequent as carcinoma of the pancreas, while it is almost the reverse in this country.

(Colonel J. E. Ash, Washington, D. C.) I am wondering if the essayist meant to imply that circumcision and sex hygiene in themselves would prevent carcinoma of the penis. It is certainly true that this tumor is almost unknown among those who practice circumcision and that it is very common in China where circumcision is not practiced and also where personal hygiene is unknown among the poorer classes. Smegma is a mild carcinogen. The important factor, therefore, is not necessarily circumcision or sex hygiene as much as it is the use of soap and water.

(Dr. Plaut) Colonel Ash mentioned the fact that smegma is a carcinogenic

substance. I do not know how he knows about that. I made the same assumption years ago. The dorsal skin of numerous mice was treated with human or equine smegma. The results are not as yet properly tabulated, but two carcinomas grew at the site of treatment, and several papillary lesions were observed. Whether the results are significant enough to call smegma an experimental carcinogenic agent I do not yet know.

**ANGIOMATOID CHANGES IN THE GENITAL ORGANS WITH AND WITHOUT TUMOR FORMATION.** Robert P. Morehead, Winston-Salem, N. C.

*Abstract.* A characteristic group of tumors of unusual structure which occur in the genital tract has been described in the literature under a variety of names. These tumors have been designated as adenomas, adenocarcinomas, mesotheliomas, mixed leiomyomas and lymphangiomas, and adenomatoid tumors. These neoplasms are characterized by vacuolization of incompletely differentiated mesenchymal cells, which ultimately results in the formation of spaces lined by cells of unusual structure. Regarding the exact nature of these cells, there is considerable difference of opinion. In this paper it is shown that these same changes take place in the genital organs apart from neoplasia, and evidence is presented to support a histogenetic relationship with vascular differentiations of the mesenchyme.

*Discussion*

(Dr. Alfred Plaut, New York, N. Y.) I fully agree with Dr. Morehead that these tumors are angiomatic, and that all the changes which make the lining cells look like epithelium are secondary. Lymphangioma in genital organs is not so rare. In my own material I have seen five in the last 10 years, and I have been shown numbers by other pathologists. There is a similarity in localization: in the epididymis in the male and in the tube in the female—certainly not a morphologic parallelism, but a physiologic one. I have no explanation for that. The lymphoid foci mentioned are not merely accumulations of lymphocytes; a reticulum stain shows a reticulum exactly as in lymphoid tissue. This, in my opinion, is another strong argument in favor of interpreting all these tumors as lymphangioma no matter how much some of the lining cells may look like epithelium. Dr. Morehead has not mentioned the most spectacular lymph-vessel tumor in the genital tract, namely, the lymphangiocystic fibroma of the uterus. This very rare tumor—I have seen only one—can reach the size of a full-term gestation.

**HISTOGENESIS OF HYDATIDIFORM MOLE.** Nicholas M. Alter, Jersey City, N. J.

*Abstract.* Within the limits of this paper only the salient facts are presented. At the Margaret Hague Maternity Hospital 60 cases of true hydatidiform mole were observed in the last 15 years among 83,225 deliveries and 2,750 abortions, giving a proportion of 1 in 1,400 pregnancies.

Case 2 illustrates the clinical and pathologic findings of a benign mole. Microscopically, villi were all without blood vessels. Over the surface anaplastic proliferation of trophoblasts was seen. The glycogen content in the benign type was marked. Mostly over the surface of Langhans' cells, syncytial giant cells were seen with vacuolated cytoplasm which often took fat stains. In the stroma regressive changes were seen with cyst formation; near the surface trophoblasts invaded the stroma and might be found at a distance.

The malignant course is typified by case 41. Malignant choriocarcinoma was found in the uterus removed 7 weeks after the mole. Upon microscopic examination a diffuse proliferation of small cuboidal cells was seen; there was no structural arrangement; necrosis and hemorrhage were extensive; the cells showed anaplasia, some mitotic figures, and pyknotic nuclei; they contained no glycogen.

Hydatidiform moles removed superficially or passed give a variety of histologic pictures depending on (1) their situation in utero, (2) regressive changes due to retention in utero, and (3) changes after removal. Hydatidiform moles *in situ* naturally can give additional information. Such a uterine specimen was removed in case 7. Frozen section of the uterus was made in order not to disturb the contents. On cross section a rather orderly arrangement was observed. Microscopic examination revealed more exuberant epithelial proliferation near the blood supply at the base than in the interior. Where trophoblasts were less proliferative and more differentiated they formed lacunae obviously for fluid exchange replacing vascularization; such channels could be observed over the surface as well as within the villi.

To extend this structural study, suitable specimens were pieced out from physiologic solution. One such preparation was 30 cm. in length. Microscopic sections of the alternating blebs and stems had epithelial covering of trophoblasts, which in places invaded the deeper layers and seemed flattened at the lining of the cavity. They formed also partly hyalinized masses within the stems. The blebs of the mole were analogous to retention cysts with retained material of metabolism due to complete lack of drainage. In material from abortions the avascular villi showed marked trophoblastic proliferation with bud-like outgrowth of branches.

**Summary.** A true hydatidiform mole is an epithelial neoplasm of the chorionic trophoblasts with secondary cystic changes of the stroma in uniovular pregnancy. This epithelial proliferation shows various degrees of the histologic changes found in malignant growth: anaplasia and invasion, which are, however, intrinsic characteristics of these embryonal cells. Evidence seems to indicate that these proliferative changes are due to the disappearance of the allantoic circulation. Cystic changes of chorionic villi due to regression of the final vascular system are false moles, as observed in early abortion, ectopic and other uniovular pregnancies.

**EXAMINATION OF SPUTUM FOR CANCER CELLS AND PARTICLES. REVIEW OF LITERATURE AND CASE REPORTS.** Siegfried Tannhauser, Buffalo, N. Y.

**Abstract.** Examination of sputum for cancer cells has been largely neglected, although results in suitable cases are surprisingly unequivocal. The examination is done in smears or, as in the cases of this paper, in paraffin embeddings of the whole sputum collected over several days in acidified formaldehyde solution, which clumps and solidifies the mucoid sputum into relatively firm matter easily dehydrated and embedded in the usual way. This method allows making serial sections of the sputum. Ten cases with positive results are presented, verified either by biopsy or autopsy. It appears that shedding of cells and particles occurs predominantly in the small-celled and alveolar types of pulmonary carcinoma. Secondary malignant neoplasms are also amenable to diagnosis, if they produce ulcerative lesions in the bronchial tree.

**CARCINOMA OF THE THYROID OCCURRING IN A CASE OF DIFFUSE TOXIC HYPERPLASIA TREATED PREOPERATIVELY WITH THIOURACIL.** A. R. Crane and (by invitation) R. L. Payne, Norfolk, Va.

**Abstract.** The marked epithelial proliferation produced in the thyroid by thiouracil and the fact that thiourea in combination with 2-acetaminofluorene produces carcinoma of the thyroid in rats raise the question as to the carcinogenic properties of thiouracil in man. Because of this the following case is reported. A white female, 56 years old, with a typical history of hyperthyroidism and showing the clinical findings of this condition, had a subtotal thyroidectomy done. Prior to operation she was treated with thiouracil over a period of 6 weeks, receiving 10.1 gm. in all. The thyroid tissue removed weighed 17.5 gm. It showed one small

(1 cm.), white, firm area in each lobe. The remainder of the gland was normally lobulated and brown. Sections of the small areas showed an irregular glandular and papillary growth with large cells, atypical nuclei, and prominent nucleoli. These have been interpreted as small areas of carcinoma. The remainder of the tissue showed a typical picture of involuting diffuse hyperplasia. The facts that thiourea in combination with 2-acetaminofluorene produces carcinoma of the thyroid in rats and that carcinoma of the thyroid is rare with diffuse hyperplasia suggest that there may have been an association between thiouracil and the development of carcinoma in this case. The validity of this hypothesis will be determined by subsequent observations.

#### *Discussion*

(Dr. Edmund Mayer, Stamford, Conn.) It is difficult to find any similarity of mechanisms in a thyroid carcinoma in a thiouracil-treated patient and the formation of those thyroid tumors which we observe in thiouracil-treated rats. In the experimental rat, thiouracil acts on a normal thyroid, while it acts on a hyperactive gland in the human patient. The production of thyroxin is depressed from normal to subnormal in the rat. As a consequence the anterior pituitary body produces more thyrotropic hormone, which has the following effect: The thyroid remains unable to correct its functional deficiency, but it can and does react with morphologic activation, first within the regular histologic pattern, and finally, after about 8 months of continuous administration, with the formation of adenomas and occasional carcinomas. In the human patient an excessive functional and morphologic activity of the thyroid is present before treatment with thiouracil. When, through this treatment, a more or less normal condition has been obtained, why should the anterior pituitary gland start to produce more thyrotropic hormone than before? If so, why should the thyroid be activated morphologically beyond its condition before treatment, and why should the histologic pattern be lost now, when it had been maintained during the highest degree of hyperthyroidism? In other words, the morphologic processes in the thyroid develop in opposite directions when a normal rat and a human patient are treated with thiouracil. The more we can be certain that the thyroid tumors in rats are caused by thiouracil, the less we can assume that a thyroid carcinoma has been caused by thiouracil treatment of a patient with hyperthyroidism.

(Dr. Oscar B. Hunter, Washington, D. C.) Have you made any cholesterol studies in this case?

(Dr. Crane) I believe that a cholesterol study was not done on this patient.

#### THE IN VIVO SENSITIVITY TO STREPTOMYCIN OF RECENTLY ISOLATED STRAINS OF HUMAN TUBERCLE BACILLI. William H. Feldman and (by invitation) H. Corwin Hinshaw, Rochester, Minn.

**Abstract.** The original observations on the ability *in vivo* of streptomycin to exert a successful deterrent effect on experimental tuberculosis were made with infections established by the laboratory strain of tubercle bacilli H 37 r v. The importance clinically of information regarding the *in vivo* efficacy of streptomycin against previously uncultured strains of tubercle bacilli obtained directly from patients with tuberculosis is obvious. Ten experiments were done using (1) seven freshly isolated strains of tubercle bacilli obtained by gastric aspiration of 7 patients with severe and progressive pulmonary tuberculosis; (2) two strains of bovine tubercle bacilli, and (3), for comparison, H 37 r v. Each strain of tubercle bacilli was used to inoculate subcutaneously 14 guinea-pigs. Starting 2 weeks later, 8 animals in each group were treated daily with streptomycin. Treatment continued for 54 days. The experiments were terminated on the 68th postinfection day. The re-

sults indicated quite definitely that streptomycin was equally effective against the infection produced by each of the ten strains of tubercle bacilli. Although practically all of the untreated controls in the respective groups showed a severe generalized and uninhibited disease, the disease in the treated animals was in most instances not demonstrable or was limited to the site of inoculation.

**THE EFFECT OF STREPTOMYCIN ON THE HISTOPATHOLOGY OF HUMAN TUBERCULOSIS.** Archie H. Bagenstoss, William H. Feldman, and (by invitation) H. Corwin Hinshaw, Rochester, Minn.

*Abstract.* There is convincing evidence that streptomycin exerts a profound regressive action on the morphologic aspects of well established tuberculosis in guinea-pigs. Material from 5 fatal cases of tuberculosis in human beings treated with streptomycin was studied to determine if similar morphologic signs of therapeutic effect could be recognized. For control purposes an unselected group of similar cases that had not been treated with streptomycin was also studied. The observations on the few cases examined suggest that following a period of treatment with streptomycin lasting several weeks or more, tuberculous lesions in the spleen, liver, and especially the lungs are more discrete and less cellular. Many of the lesions become atrophic, fibrotic, and hyalinized. Caseation is minimal or absent. In many instances the lesions eventually lose their characteristic tuberculous identity and appear as nonspecific granulomatous foci.

The findings in the central nervous system were unique. The meningeal lesions in some cases were regressing or were no longer demonstrable, although in some instances an apparently uninhibited, focal tuberculous encephalitis was present. The absence of meningeal lesions in cases in which tubercle bacilli had previously been demonstrated in the spinal fluid would seem to be a significant observation.

*Discussion*

(Dr. Kornel L. Terplan, Buffalo, N. Y.) The hematogenous tubercle has, in general, the tendency to heal. In cases with miliary tuberculosis as the cause of death we see a good number of hematogenous tubercles in the liver or in the lungs with a very marked healing tendency. Only in the massive overwhelming type of acute miliary tuberculosis, especially in very early life, combined with hemorrhages in casedated lesions of the primary complex, are the hematogenous tubercles actually miliary necroses. In the majority of children with miliary tuberculosis a healing tendency of the hematogenous tubercle in various organs could be noticed. The cause of death in most of these was tuberculous meningitis. It might be of interest to evaluate the results described by Dr. Feldman and associates in connection with the duration of the disease and the type of infection (whether acutely overwhelming or the more usual type of progressive miliary tuberculosis).

(Dr. Howard C. Hopps, Oklahoma City, Okla.) I should like to ask why so many of the experimental animals that received streptomycin died from causes which were apparently not related to tuberculosis.

(Dr. Feldman) That is a good question, and I hoped that some one would ask it. We lost some, it is true. Most of these animals had at necropsy a massive abdominal hemorrhage. We were quite concerned for a while, and were at a loss to account for these premature deaths. We finally thought of the possibility that these animals may have been subjected to injudicious restraint when they were being injected with streptomycin. We drew this to the attention of our assistants. Since then few deaths from abdominal hemorrhage have occurred. This may or may not be the answer.

(Dr. William H. Harris, New Orleans, La.) I would like to ask whether this was

a "prophylactic" experiment, or was it established that such animals had already developed the disease.

(Dr. Feldman) I tried to make it clear that we injected the animals with 0.1 mg. of the organism and waited 2 weeks before treatment, and then they were treated daily with 6,000 units of streptomycin given in divided doses. On this basis it was a therapeutic and not a prophylactic experiment.

(Dr. Harris) Were any of the animals of the group to be treated sacrificed at the 2-week interval in order to ascertain the degree of lesion present at that period?

(Dr. Feldman) One or two of them had died with tuberculosis of the liver or spleen at that time; and even those which died within 10 days showed microscopically some tuberculosis of the spleen.

**INFLUENCE OF PENICILLIN IN SUBACUTE BACTERIAL ENDOCARDITIS.** Robert A. Moore, St. Louis, Mo.

*Abstract.* Healing in subacute bacterial endocarditis includes four basic processes: covering the vegetation by fibrous tissue; phagocytosis and destruction of the bacteria; organization and calcification of the necrotic central part of the vegetation; and endothelialization of spaces within the vegetation. Penicillin, by controlling the further growth of bacteria, allows healing to occur more rapidly. Treatment should include other agents known to influence wound healing, notably maintenance of an adequate level of plasma albumin and a sufficient intake of vitamins.

*Discussion*

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) I would like to ask Dr. Moore if he has seen recurrence after treatment. The reason I ask is that I have seen several cases which have been treated and clinically were apparently cured. The blood stream became free and all symptoms disappeared; and 2 patients have died from other causes such as heart failure or mycotic aneurysms in the brain. In those heart valves I found, as he did, that the organisms were gone. The valves were in the process of healing. In some of my cases I have found a typical picture of acute rheumatic endocarditis of the valve. That led me to believe that we have a rich field there for re-infection and re-occurrence of bacterial endocarditis. I have not seen recurrence in the few cases I have observed.

(Dr. Moore) I cannot answer Dr. Clawson's question, inasmuch as my experience with this disease is largely limited to the study of the pathologic anatomy of the lesions of the heart. However, in the Barnes Hospital I can say that there have been a number of patients with recurrences similar to those cited by Dr. Clawson. I have not observed in these 24 cases any evidence of active rheumatic fever after the process of healing had occurred.

(Dr. Howard T. Karsner, Cleveland, O.) Experience has shown that in clinical practice large doses of penicillin are necessary for satisfactory treatment of subacute bacterial endocarditis. Did all the patients reported by Dr. Moore receive doses of penicillin which, at the present time, would be regarded as therapeutically adequate?

(Dr. Moore) No, Dr. Karsner, they did not. Some of the patients treated in 1942 received relatively small amounts of penicillin. From a study of these slides it is difficult to make any definite recommendation as to the dosage, but I may say this: A dose of penicillin should be given which will maintain a blood level to which the organism in that patient is sensitive over a sufficient period of time to bring about healing of the vegetation. That is the general principle. To be more specific, I think the dosage is never less than 200,000 to 400,000 units in 24 hours, given intravenously or intramuscularly at least every 2 hours, and for a period of time somewhere between 1 and 4 months.

**THE EFFECTS OF RADIOACTIVE PHOSPHORUS ( $P_{32}$ ) ON THE MALIGNANT LYMPHOMAS.** William R. Platt (by invitation), St. Louis, Mo.

**Abstract.** A study of the cellular changes occurring in the tissues of 35 patients treated with radioactive phosphorus is reported. The various types of leukemia constitute the majority of the cases studied. However, several lymphosarcomas and leukosarcomas, one case of Hodgkin's disease, one Ewing's tumor, and one melanoma were also included in the series. Quantitative measurements of residual  $P_{32}$  in the autopsied organs are correlated with the histopathologic changes observed.

**Discussion**

(Dr. Howard T. Karsner, Cleveland, O.) The possibility of injury by the use of  $P_{32}$  as indicated by Dr. Platt's report, together with reports of lack of benefit in leukemic disorders by treatment with  $P_{32}$ , raise the question as to whether this agent may do more harm than good.

**THE EFFECT OF BAL THERAPY ON THE PATHOLOGY OF SYSTEMIC CADMIUM POISONING.** Arthur M. Ginzler, Maj., M.C., and (by invitation) A. Gilman, Maj., Sn.C., F. S. Philips, 1st Lt., Sn.C., R. P. Allen, and E. S. Koelle, Edgewood Arsenal, Md.

**Abstract.** The development of BAL (2, 3-dimercaptopropanol) has led to great advances in the understanding of the toxicologic and pharmacologic mechanisms involved in heavy metal poisoning. Evidence has accumulated that the therapeutic action of BAL depends on its ability to compete for the intoxicating metal against vital SH-containing protoplasmic constituents, apparently enzymatic proteins, with the formation of relatively nontoxic mercaptides.

Although cadmium poisoning is not of common clinical occurrence, it notably exemplifies the mechanisms involved in this group of agents as a whole. The intravenous administration to rabbits of lethal amounts of cadmium chloride results in acute toxic death, in most instances within 24 hours, accompanied by moderate central and midzonal hepatic necrosis, lymphorrhesis, visceral congestion, and, with rare exception, *insignificant or no renal lesions*. In striking contrast, when the injection of the cadmium salt is accompanied by the prophylactic administration of BAL (1 minute prior to the cadmium), there is an exaggeration of the hepatic and lymphoid damage, and more important, the development of severe renal damage manifested by extensive tubular necrosis, chiefly of the proximal convoluted tubules. Gilman *et al.* (in press) have explained these effects on the basis of an *in vivo* 1:2 combination of cationic cadmium and BAL to form a soluble  $Cd(BAL)_2$  complex, excretion of which is more greatly (or more rapidly) effected by the kidney than is that of cadmium in untreated animals; they postulate that *in vivo* mechanisms result in dissociation of this complex within the renal tubular epithelium with the consequent accumulation within the kidneys of far greater amounts of cadmium than is the case in the untreated rabbits. They have submitted evidence for the actual *in vitro* formation of such a complex, as well as another, insoluble, 1:1  $Cd$ -BAL complex. Further information has been gathered from the pathologic effects of injection of the actual preformed complexes; the pathology of the insoluble  $Cd$ -BAL resembles that of untreated cadmium, whereas the pathology of this complex plus the prophylactic administration of BAL, as well as the pathology of the soluble  $Cd(BAL)_2$  with no further treatment, resembles that of cadmium plus prophylactic treatment with BAL.

Actually, therapeutic administration of BAL (at an interval after the injection of the cadmium salt), as compared with the prophylactic administration of BAL, results in definite amelioration of both the hepatic and renal lesions. A proffered

explanation (Gilman *et al.*) is that the delay in therapy may permit the rapid irreversible tissue fixation of nontoxic amounts of cadmium. Such a mechanism would result in the mobilization of lesser amounts of cadmium to the kidneys and liver, as compared with prophylactic treatment, and consequent lesser degree of injury to the kidneys and liver.

**ON THE NATURE AND GENERAL PATHOLOGIC SIGNIFICANCE OF GRANULOMATOUS INFLAMMATION.** Wiley D. Forbus,\* Durham, N. C.

*Abstract.* In this paper the author deals with a general pathologic consideration of the subject of granulomatous inflammation, with particular emphasis upon the specific chemotactic properties of certain etiologic factors for the cells of the reticulo-endothelial system. A reorientation of the pathogenic problems involved in the granulomatous diseases, particularly those produced by some of the more important fungi, is attempted. A brief statement of the general pathologic significance of granulomatous inflammation is given in the concluding section of the paper, which will be published in full in the American Lecture Series, published by Charles C. Thomas.

**TISSUE CHANGES IN FUNGUS DISEASES.** Roger D. Baker, Birmingham, Ala.

*Abstract.* When the microscopic appearances of fungus diseases were tabulated with respect to the degree of suppuration, macrophage and giant cell response, caseous necrosis and fibrosis, the following observations were made:

1. Several of the deep fungus infections, such as blastomycosis (North and South American), coccidioidomycosis, sporotrichosis, and moniliasis (when a deep infection), show all of these tissue changes.
2. Others of the deep infections, such as actinomycosis, nocardiosis, and maduromycosis, show all of these changes except caseous necrosis.
3. A few of the deep mycoses are not usually attended with suppuration.
4. Mycoses may run their entire course with only acute necrosis or acute inflammation.
5. The superficial fungus infections often have no inflammatory response, but may have acute or chronic inflammatory response.

It is concluded that chronic suppuration with fibrosis is probably the most general tissue change in deep fungus infections and that the neutrophil is more usually the primary reacting cell. In some instances, however, the macrophage and giant cell may be the primary reacting cells. Tissue changes in fungus infections represent response to proliferating and dying foreign bodies, probably in some instances together with hypersensitivity to the presence of the endotoxin.

**BRUCELLOTIC OSTEOMYELITIS OF ILIUM AND SCAPULA WITH GRANULOMAS OF LIVER AND GALLBLADDER.** Leo Lowbeer (by invitation), Tulsa, Okla.

*Abstract.* A 63-year-old dentist contracted brucellosis by drinking raw milk from aborting cows, apparently infected through contact with lame brucellosis hogs. Brucellosis presented a chronic, recurrently febrile course for 2½ years, after which time soft tissue "abscesses" gradually developed, one in the left gluteal, one in the sacral, one in the right inguinal, and one in the left axillary region. Roentgenograms showed destructive lesions in both alae ilii and in the left scapula. Incision of all four abscesses yielded cheesy purulent material from which *Brucella suis* could be grown in pure culture. Inoculation of a guinea-pig resulted in typical brucellosis abscess-like granulomas in the internal organs within 8 weeks, from which

\* By invitation of the Council.

*Brucella suis* could be cultured. The strain was found penicillin-susceptible in a concentration of 1.4 units per cc. Microscopic examination of the necrotic material showed granulation tissue with acidophilic histiocytes, occasional giant cells, lymphocytes, extensive necrosis, destruction of the cortex of the os ilii, and osteosclerosis alternating with osteoporosis. The abscesses drained for many months despite penicillin treatment. One year later the patient was operated upon for symptoms of cholecystitis; a grossly normal appearing gallbladder was removed and a specimen was taken for biopsy from a grossly normal liver. The liver showed many round granulomas, which were also found in the wall of the gallbladder. The bile was sterile. Several months later the sinuses were still draining and yielding *Brucella suis* which caused fatal purulent Brucella peritonitis and septicemia in inoculated guinea-pigs within 1 week. Roentgenograms of the chest showed multiple shadows in the right lung and effusion in the left pleura, found to be clear exudate. Bone lesions are unchanged in the roentgenograms. The strain was found to be inhibited by streptomycin in concentration of 1 unit per cc.

The case is presented (a) to demonstrate again the granulomatous nature of brucellosis which, although known in the guinea-pig since the investigations of Theobald Smith and Fabyan in 1912, has been shown to exist in man only during the last decade on the basis of a few necropsy reports; (b) to demonstrate the virulence of the porcine strain with its tendency to necrosis and "abscess" formation; (c) to show for the first time the microscopic appearance of brucellosis osteomyelitis in man, which previously had been diagnosed only on the basis of clinical, roentgenologic, and bacteriologic findings.

*Note.* Cultures were checked by Dr. I. H. Borts, Iowa State Laboratory; penicillin assay by Dr. C. S. Keefer, National Research Council; slides from bone, liver, and gallbladder by Dr. W. D. Forbus.

#### GRANULOMATA OF UNKNOWN ETIOLOGY ASSOCIATED WITH PERIARTERITIS NODOSA.

REPORT OF TWO CASES. Tobias Weinberg, Baltimore, Md.

*Abstract.* The first case is that of a male, aged 38, with a history of sinus trouble for 10 years. He developed bronchiectasis for which a lobectomy was performed 4 years before death. He was admitted to the hospital because of bleeding from the nose, and was found to have numerous ulcers of the nose and mouth. He died suddenly 3 weeks after admission. The autopsy revealed ulcerations in the mouth, trachea, and bronchi; large scattered areas of consolidation in both lungs; splenomegaly with areas of infarction, and swollen kidneys containing numerous petechial hemorrhages and scattered areas of infarction. Microscopically, the areas of consolidation in the lungs showed a chronic granulomatous reaction containing areas of necrosis and numerous giant cells. In most instances they surrounded blood vessels. These and other blood vessels showed a typical picture of periarteritis nodosa. Similar granulomata were found in the trachea, bronchi, spleen, tongue, and kidneys. Periarteritic lesions were also present in the spleen and kidneys.

The second case was a female, aged 50, who gave a history of pain in the legs and arms of 3 weeks' duration. There was a history of pleurisy 6 months before. Physical examination revealed a maculopapular rash over the arms, face, and trunk. The patient died 3 days after admission. Autopsy revealed lesions in the lungs similar to those in case 1. The kidneys were swollen and exhibited numerous petechial hemorrhages. A ruptured aneurysm of a branch of the renal artery was present near the lower calyx of the left kidney. Microscopically, the picture was the same as in case 1, but showed periarteritic lesions in the heart as well as in the lungs, spleen, kidneys, and gallbladder. Granulomatous lesions were present in the lungs, liver, spleen, and kidneys.

No bacteria were stainable in the granulomata in either case, nor have fungi or parasites been demonstrated. The remaining possibility is that a virus is the causa-

tive agent. Periarteritis nodosa is apparently a terminal development, lending support to the thesis of hypersensitivity as the cause of periarteritis nodosa.

#### *Discussion*

(Dr. G. Lyman Duff, Montreal, Que.) Dr. More, Dr. McMillan, and I have recently studied a series of 375 autopsied cases in which it was known that considerable doses of the sulfonamides had been given during life, with a view to searching for lesions that might be attributed to the sulfonamide therapy. In 22 of the 375 cases, lesions were found of a kind that I shall mention in a moment which were attributable, we felt, to the sulfonamide therapy on the grounds of exclusion of other possible etiologic agents and on the basis of identity between the lesions in the 22 cases studied and the lesions described in experimental animals treated with sulfonamides. The most frequent lesion was a granulomatous one which was encountered in 13 of the 22 cases. It was found most often in the heart, liver, or kidneys, less frequently in other organs. In one case only microscopic granulomas were found in the lung and in the wall of a bronchus. Such granulomatous lesions have been described before as being associated with sulfonamide therapy. They have also been produced in experimental animals by the administration of sulfonamides. In 7 of our cases an acute necrotizing arteritis was found. This, too, has been ascribed by Rich to sulfonamide sensitivity. Amongst the 22 cases, 4 had both lesions in common: the granulomatous lesions and the necrotizing arteritis, closely comparable with periarteritis nodosa. Whatever may be regarded as the etiology or pathogenesis of these lesions, it seems to me that this evidence gives strong support to the idea that the granulomatous reaction encountered in some cases of periarteritis nodosa belongs to the same disease or, rather, is part of that disease. Our observations also support the view that all of these lesions are of allergic nature. In any event, we have interpreted them in that sense.

(Dr. William Boyd, Toronto, Ont.) I am glad that Dr. Duff has referred to the association of the granulomatous lesions with arteritis, or, rather, with arterial lesions of various kinds, especially allergic in type. I also have seen examples of granulomatosis in sulfonamide poisoning. Recently I have encountered a case of disseminated lupus erythematosus with very characteristic collagenous degeneration in the glomerular tufts and also in the vessels of the lung, and in association with these changes in the lung there were beautiful granulomata extremely like sarcoid lesions. These were situated in relation to arteries. This general discussion of Dr. Weinberg's paper would suggest the addition of allergic granulomata to Dr. Forbus' classification, although they might be included in the third group of unknown etiology.

(Dr. Weinberg) I would like to point out that cases showing the same granulomatous reaction with particular involvement of the lungs and upper respiratory tract and unassociated with periarteritis nodosa have been reported in the literature as far back as 1906. Furthermore, the cases reported in 1939 by Wegener, in which the granulomatous reaction was associated with periarteritis nodosa, did not receive any sulfa drug therapy.

#### PERINEURITIC AND POLYMYOSITIC GRANULOMATOUS NODULES IN RHEUMATOID ARTHRITIS. Gabriel Steiner, Detroit, Mich.

*Abstract.* Seen from a pathologic viewpoint, rheumatoid arthritis is a misnomer. In this disease arthritic changes may be very spectacular *clinically*, but *nosologically* they do not represent the most significant aspect of the disease. The pains and the muscular atrophy in cases of rheumatoid arthritis, as well as the peculiar bilaterally symmetrical distribution, point to an involvement of the peripheral nervous system

and of the skeletal muscles. So a few years ago, upon the suggestion of Dr. Hugo Freund, an anatopathologic investigation of the nervous system of cases of rheumatoid arthritis was inaugurated. By courtesy of Dr. S. E. Gould, Wayne County General Hospital, and of Dr. Plinn F. Morse, Harper Hospital, autopsy material (peripheral nerves and muscles) and skeletal muscles taken for biopsies were obtained. Altogether 20 cases of typical rheumatoid arthritis were examined.

The peripheral nerves and muscles examined were brachial plexus, ilio-inguinal, femoral, and tibial nerves; deltoid, triceps, gastrocnemius, and rectus abdominis muscles. The nodules were sharply circumscribed. They were located exclusively in the perineurium of peripheral nerves and in the perimysium of the skeletal muscles. The cells composing the nodules were lymphocytes and plasma cells, the latter located more in the periphery. There were some epithelioid cells or macrophages, but no giant cells or Anitschkow's myocytes. In some cases a definite muscular atrophy as a consequence of the inflammatory nodules was seen. There was a marked increase of collagenous connective tissue, but proliferation of reticulin fibers was not seen. Occasionally a perineuritic nodule was seen around an intramuscular nerve bundle. Inflammatory infiltration of vascular walls was rare and seen only in the perimysium.

In summary, three points should be emphasized:

1. The nodular inflammation is not of a pure granulomatous type in so far as epithelioid cells or macrophages are inconspicuous and there is no necrosis in the center. Collagenous connective tissue proliferation is seen between the inflammatory cells (lymphocytes and plasma cells).
2. The specificity of the findings in the peripheral nerves and muscles has been established by a great number of control examinations (autopsy material, amputations, muscles taken for biopsy).
3. The nodules in the perineurium of the peripheral nerves and in the perimysium of the skeletal muscles represent a new link in the chain of lesions which indicate the *systemic* nature of the disease.

#### *Discussion*

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) Stimulated by the work of Dr. Steiner and his colleagues, I was able to get specimens for biopsy from the deltoid muscles of about 70 cases of chronic rheumatoid arthritis, and in more than half of these cases I found lesions similar to those which he described. I also was able to get muscles from a few cases of acute rheumatic fever and found the lesions in them, and likewise in one case with a healed rheumatic valvular deformity I found the lesion. I thoroughly agree with Dr. Steiner that the term rheumatoid arthritis should include and mean more than the arthritis; it should include myositis or fibrositis.

(Dr. B. Black-Schaffer, Durham, N.C.) We have had an opportunity to study a case of dermatomyositis and scleroderma recently, and I would like to point out that very similar lesions were found in both instances. In a case of myasthenia gravis associated with thymoma we also found collections of lymphocytes in the skeletal muscles.

(Dr. Steiner) Dr. Black-Schaffer spoke of having examined one case of dermatomyositis. In our control material of over 300 cases we had only one case of dermatomyositis, certainly not enough to establish a clear-cut conclusion for differentiation. However, in our one case the distribution of the inflammatory process was diffuse and not nodular. This process was spread from the extramuscular tissues into the muscles. The perineurium was not involved. If this difference could be found in every case, it would be easy to differentiate the pathologic picture of dermatomyositis from that of nodular polymyositis in rheumatoid arthritis.

We had two cases of myasthenia gravis in our control material: In one we did

not find any lymphocytic infiltration, and in the other we found some—and this is the difference—located between the individual muscle fibers, and even between individual muscular fibrillae. Another difference is the peculiar nodular type in rheumatoid arthritis contrary to the less demarcated type in the lymphorrhages of myasthenia gravis. A third difference is that the lymphorrhages are composed only of lymphocytes; there are no plasma cells, no macrophages, and no connective tissue proliferation such as are seen in the muscle nodules of rheumatoid arthritis.

**THE VENEREAL GRANULOMAS OF THE PENIS.** N. B. Friedman, Capt., M.C., and J. E. Ash, Col., M.C., Washington, D. C.

*Abstract.* Although the histologic appearance of the primary penile venereal granulomas is fairly characteristic, it is not generally appreciated that microscopic examination of these lesions may contribute information of diagnostic value.

Granuloma inguinale bears a superficial resemblance to syphilitic chancre, but the presence of many small clusters of polymorphonuclear leukocytes amidst a dense infiltrate of round cells is strongly suggestive of granuloma venereum. Donovan bodies, which are recognizable in routine hematoxylin and eosin preparations, are rendered conspicuous if the section is impregnated with silver. The bodies must be differentiated from the granules of mast cells, especially when a Giemsa stain is employed.

The tuberculoid granulomas of lymphopathia venereum are not as unmistakable when they occur on the penis as when they are encountered in the inguinal lymph nodes, but aggregations of epithelioid and giant cells in an inflammatory lesion of the penis, especially when arranged about irregularly shaped necrotic or suppurative foci, should be strongly suggestive of lymphogranuloma inguinale.

Chancroid has a less specific histologic appearance than the other penile granulomas. The early lesion is an edematous plaque, superficially ulcerated and covered by a thin layer of leukocytes, débris, and organisms. The zone beneath shows a disproportionately scanty infiltration of inflammatory elements which tend to aggregate about blood vessels. In older lesions angiitis and periangiitis dominate the picture; the lamellated perivascular and intramural rings of round cells and the absence of accompanying diffuse infiltration are characteristic of late chancroid.

**THE HISTOLOGIC DIAGNOSIS OF CHANCRON AND LYMPHOGANULOMA VENEREUM AS SEEN IN SPECIMENS FOR BIOPSY FROM GENITAL LESIONS.\*** Walter H. Sheldon and (by invitation) Albert Heyman, Atlanta, Ga.

*Abstract.* A high incidence of chancroid and lymphogranuloma venereum has been found in the general Negro hospital population of Atlanta. The incidence of these infections as compared to syphilis is about 40 per cent. A large-scale study of the clinical and laboratory aspects of chancroid and lymphogranuloma venereum was recently completed in our clinic. Biopsy and all other known diagnostic procedures such as cultures, smears, auto-inoculations, and skin test were employed in this investigation. Our histologic observations of chancroid and lymphogranuloma venereum were based upon cases proved either by culture of the Duxrey bacillus or isolation of the virus of lymphogranuloma venereum. Zenker's fixative and the phloxine methylene blue stain were used. The material from proved lymphogranuloma venereum was fixed in Regaud's fluid and stained with Giemsa's mixture.

It became apparent during this study that chancroid could be diagnosed by its histologic appearance alone. The lesion of chancroid shows a shallow ulcer with

\* See also: Sheldon, W. H., and Heyman, A. Studies on chancroid. I. Observations on the histology with an evaluation of biopsy as a diagnostic procedure. *Am. J. Path.*, 1946, 22, 415-425.

some purulent exudate beneath which two quite distinct zones of cellular infiltration are seen. The deep layer shows a dense infiltration of plasma cells and lymphocytes. There are numerous newly formed blood vessels which are arranged in palisading fashion. They show marked endothelial proliferation and, near the surface, degeneration of the vessel wall and thrombosis are commonly encountered. The tissue beneath the ulcer is cellular and consists chiefly of endothelial cells. These are found in all stages of proliferation and outnumber all other cells. The lack of appreciable proliferation of fibroblasts in the same area is striking and constitutes an important finding.

The primary lesion of lymphogranuloma venereum was studied in several proved cases. The histologic picture of this lesion is not well known, although the appearance of the lesions in lymph nodes has been described. The primary lesion is a shallow ulcer surrounded by an area of diffuse inflammatory cellular infiltration in which large mononuclear cells predominate. These form occasional small granulomatous foci which display central necrosis. Neutrophilic polymorphonuclear leukocytes fill the necrotic areas. These granulomata are similar to those seen in the lymph nodes. They are formed by proliferation of large mononuclear cells in the media and the adventitia of small blood vessels. This proliferation leads to the compression and disappearance of the vessel lumen, but there is no significant endothelial overgrowth. Solid collections of large mononuclear cells appear and become confluent. The occlusion of vessels leads to central necrosis and eventually to the formation of the ulcer.

It was found that the histologic picture of chancroid was sufficiently distinct to permit a reasonably certain diagnosis and to differentiate this infection from other venereal lesions. The diagnosis of chancroid by histologic examination was far more accurate than by smear. It was more reliable and a simpler method than culture which requires special technic and considerable experience. Among all diagnostic procedures, biopsy was the most efficient single method of diagnosis. Biopsy is not practical in the diagnosis of lymphogranuloma venereum. The primary lesion is evanescent and the removal of lymph nodes is not advisable. The histologic picture of the infection is, however, sufficiently distinct to permit the diagnosis. We have found that chancroid, lymphogranuloma venereum, syphilis, and granuloma inguinale as well as nonspecific genital lesions can be diagnosed by histologic examination with a high degree of accuracy. In our experience histologic examination of genital lesions offers more information than any other single diagnostic procedure.

**DISSEMINATED GRANULOMA VENEREUM.** John S. Howe and (by invitation) M. Markowitz, Richmond, Va.

**Abstract.** Granuloma venereum can no longer be considered a disease entirely localized to external genitalia and inguinal regions. Extranodal lesions occur in 6 per cent of cases, usually attributed to contact or auto-inoculation. Lesions of the cervix uteri have been described by several authors, and Pund and associates have described involvement of uterus, tubes and ovaries in 4 cases. Reports of more widely disseminated lesions have been few, and few necropsies have been reported.

We have observed at autopsy a case of extensive disseminated granuloma venereum in a 27-year-old Negress with rapid progression to a fatal outcome following pregnancy. The primary lesions were specific ulcers of cervix and vagina, followed by extension to the endometrium, myometrium, tubes and ovaries, rectum and bladder. There was extension by lymphatics to the iliac and lumbar lymph nodes and iliopsoas muscles bilaterally. There were also localized lesions of the cecum with involvement of the ileocolic lymph nodes, localized specific nodules in both kidneys, and a specific left pretibial ulcer, all probably due to retrograde lymphatic

spread. Microscopically, all lesions showed foamy macrophages predominating, nearly all of which contained numerous intracellular Donovan bodies. Plasma cells were present in moderate numbers, and a few polymorphonuclear leukocytes were seen, some of them eosinophilic. Areas of fibrosis were present, particularly in the iliopsoas muscles. This appears to be the first confirmation of the reports of Pund and associates of granuloma venereum involving tubes and ovaries, and apparently records for the first time involvement of kidneys and iliopsoas muscles by granuloma venereum.

#### *Discussion*

(Dr. Walter H. Sheldon, Atlanta, Ga.) We have seen many instances of granuloma inguinale (granuloma venereum) and have encountered several cases of this disease which showed systemic manifestations. Last year we reported a case of osteomyelitis of the tibia caused by granuloma inguinale. Since then we have seen a patient with granuloma inguinale involving the trachea and apparently the lungs. There was a tracheal fistula. Tissues taken for histologic study from the tracheal fistula and from a lower level of the trachea showed granuloma inguinale. The lungs showed extensive involvement on roentgenologic examination. At present we are studying a case in which there was extensive granuloma inguinale of the perineum 1 year ago. The perineal lesion had healed, but at autopsy multiple abscesses were found in the lungs and in one kidney.

It might be permissible here to emphasize another aspect of this disease. Extranodal lesions which are not necessarily systemic manifestations are quite frequent but unfortunately are not diagnosed, simply because they are not suspected. I have in mind the case of a man with a lesion on the lip, which is a common site of extragenital infection. For over 1 year this man had been treated vigorously for cancer. After the correct diagnosis was made, proper therapy caused healing of the lesion.

#### DEVELOPMENT AND PATHOGNOMONIC EVALUATION OF THE STERNBERG-DOROTHY REED CELL. Fritz Levy, Elkins, W. Va.

*Abstract.* This is the first exhibition of a complete series of photomicrographs explaining the development of the Sternberg-Dorothy Reed cell. These plurivalent cells arise as typical blind alleys of development by pluripolar mitoses.

The exhibit shows: (1) photomicrographs of Sternberg-Dorothy Reed cells and comparable occurrences in other tissues; (2) diagrams of types of aberrant mitoses; (3) geometric comparison of sizes of nuclei in spheroidal and flat cells with valences 1:2:4.

The comparison with similar occurrences proves:

1. The Sternberg-Dorothy Reed cell develops principally in the way that we recently described for the development of the megakaryocytes of the bone marrow. We confirmed with new photomicrographs of megakaryocytes the findings formerly described with drawings by Heidenhain, myself, and Wuyts.
2. Isolated pluripolar mitoses occur in every kind of tissue when a cell division, especially a cleavage of the cytoplasm, is temporarily disturbed (by ether, colchicine, cold, some hydrocarbons, etc.).
3. Not uncommonly, pluripolar mitoses are found in tissue where a rapid cell division occurs in a small area. In normal human and animal tissue this is found exclusively (a) in the bone marrow, leading to the formation of megakaryocytes, (b) in spermatogenesis (best observed seasonally, *e.g.*, in frogs), (c) in regenerating liver tissue.
4. Numerous pluripolar mitoses leading to the formation of plurivalent giant cells are found in rapidly growing tissue, as in many malignant tumors.

5. If the volumes of cells are in relation 8:16:32, then the radii of nuclei are, in spheroidal cells, 12:16:20; in flat cells, 16:23:32.

6. Nucleoli are especially flat parachromosomal formations. They, therefore, increase enormously in size in plurivalent cells.

7. The Sternberg-Dorothy Reed cells are more or less exactly 4, 8, 16, 32 valent cells of a tumor of flat cells, most probably a reticulo-endotheliosarcoma (Hodgkin's sarcoma, Karsner).

VISCERAL LESIONS OF ACUTE INFECTIOUS MONONUCLEOSIS. A REPORT OF TWO CASES WITH FATAL SPONTANEOUS RUPTURE OF THE SPLEEN. John H. Fisher, London, Ont.

*Abstract.* Heretofore, knowledge of the pathology of acute infectious mononucleosis has been derived chiefly from examination of the blood and bone marrow during life and from a study of excised lymph nodes. Extremely few opportunities have been afforded to study the disease process in the body as a whole at autopsy. Van Beek and Haex (*Acta med. Scandinav.*, 1943, 113, 125) have described lesions in the liver as seen in material obtained by aspiration from living patients. They claim that liver changes in mononucleosis have not been recorded previously.

Two cases are here reported in which death occurred from spontaneous rupture of the spleen. This is a very rare complication. Clinically and pathologically they were almost parallel cases. Both were young Canadian soldiers observed at autopsy in the Medical Services of the Canadian Army Overseas. Widespread accumulations of abnormal lymphocytes (mononucleosis cells) were found in most of the viscera. The lesions in the liver were similar to those described by Van Beek and Haex. Superficially, they reminded one of the liver in leukemia and also showed some resemblance to the liver lesions in the early stages of acute infectious hepatitis. The lymph nodes and spleen showed intense diffuse infiltration of their pulp with mononucleosis cells. The bone marrow was normal. The lesions of infectious mononucleosis as seen in these two cases are compared with those of acute lymphatic leukemia. Points of similarity between the disease process in acute infectious mononucleosis and that in acute infectious hepatitis are discussed.

*Discussion*

(Dr. R. H. Rigdon, Little Rock, Ark.) This spontaneous rupture of the spleen is very interesting. Sometimes we see a similar lesion in malaria. I have had opportunity to study the spleen in malaria and have found a proliferation of the cells in the splenic pulp projecting into the large sinuses. This produces mechanical blockage of the circulation. It has been suggested that this may be the basis for rupture of the spleen. It would be interesting to know whether or not you have observed such a proliferation of the cells in the splenic pulp in these cases of infectious mononucleosis. A similar process can be seen in cases of leukemia.

(Dr. Paul R. Cannon, Chicago, Ill.) Major Custer told me recently that they have seven cases of mononucleosis with rupture of the spleen at the Army Institute of Pathology, and suggested the possibility that in some instances palpation of the spleen might precipitate rupture. It should be noted, also, that in the older literature there are a number of probable cases of acute infectious mononucleosis with rupture of the spleen which have been reported as acute Hodgkin's disease.

(Dr. Horace K. Giffen, Youngstown, O.) Very recently our roentgenologist more or less incidentally made a roentgenogram on a case of acute infectious mononucleosis and found a picture which was reminiscent of the infiltration in psittacosis. Neither he nor I could find in the literature any suggestion of pulmonic lesions. I wonder if anybody else has x-ray findings on these cases.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) I would like to ask the speaker whether

there were, in the two cases he has seen, any clinical symptoms pointing to meningeal involvement. In a case with rupture of the spleen which I had occasion to examine a few months ago, the clinical diagnosis leaned first strongly towards some peculiar basilar meningitis with involvement of the nerves of the ocular muscles. The patient recovered. The gross and histologic findings in the spleen, examined by me, were in line with the very few reports known from the literature.

(Dr. Fisher) In reply to Dr. Rigdon's question, I should say that in the literature there are many cases of spontaneous rupture of the spleen in malaria. In my two cases of mononucleosis the splenic sinuses contain numerous mononucleosis cells, and the splenic pulp is packed with similar cells which compress and narrow the sinuses. Concerning vascular obstruction, I cannot say more than that. However, in the two cases I am reporting I believe that there is no evidence that hemorrhage occurred within the splenic substance but rather that it commenced as subcapsular hemorrhage, gradually stripping off the capsule which finally ruptured, resulting in fatal intraperitoneal hemorrhage.

I should like to thank Dr. Cannon for bringing to my attention the seven cases that Dr. Custer is now collecting. I shall be much interested in seeing his report. In my first case the man died on a day on which medical ward rounds were held in the morning. No doubt his spleen was palpated several times by the attending staff. We considered this as a possible factor in promoting rupture of the spleen.

In reply to Dr. Giffen I would point out again that in mononucleosis there are pulmonary lesions, particularly in the perivascular and peribronchial connective tissue, but I do not know whether these lesions produce any demonstrable roentgenologic findings. I have seen no reference in the literature to such findings.

In reply to Dr. Terplan, no evidence of meningeal involvement was noted in either of my cases. Unfortunately, the brain was not examined in either case. However, meningeal involvement is fairly commonly encountered in mononucleosis and many such cases are now appearing in the literature.

**SYSTEMIC NONLIPOID RETICULO-ENDOTHELIAL GRANULOMA (LETTERER-SIWE'S TYPE): A PATHOLOGIC STUDY OF FOUR CASES.** Louisa E. Keasbey (by invitation) and William O. Russell, Los Angeles and Santa Barbara, Calif.

*Abstract.* This disease, observed in early childhood, is characterized by marked generalized hyperplasia of histiocytes, causing splenomegaly, generalized enlargement of lymph nodes, infiltrations in the skin, tumor-like replacement of bone, and a fatal anemia. The relation of the disease to infectious reticulosclerosis, the infectious granulomas, and the diseases of disturbed lipoid metabolism is discussed.

**DIFFERENTIATION OF LEUKEMIAS AND DISORDERS OF THE LYMPHATIC APPARATUS BY LEUKO-AGGLUTINATION.** Bernhard Steinberg and (by invitation) Ruth A. Martin, Toledo, O.

*Abstract.* Various types of circulating cells and some of the fixed tissue cells were differentiated by agglutination with specific antisera. Mature and immature lymphocytes and granulocytes were found to possess a cell specificity. On that basis, leuko-agglutination (clumping of circulating or fixed tissue cells by antibodies or similarly acting substances) was used to differentiate the various types of leukemia and lymphoblastoma. Normal peripheral leukocytes were antigenically similar to leukemia. Leukemic leukocytes were antigenically dissimilar from lymphosarcoma (reticulum cell type and malignant lymphocytoma, Ewing), and leukosarcoma. On the other hand, leukosarcoma (Sternberg) and lymphosarcoma were antigenically similar. Monocytic leukemia was distinct from the other forms of leukemia, but showed a group relationship to the lymphocyte.

**GAUCHER'S DISEASE: HISTOCHEMICAL DEMONSTRATION OF KERASIN IN TISSUE.**  
Joseph Kahn (by invitation) and Abraham R. Kantrowitz, Brooklyn, N. Y.

**Abstract.** Crystals of kerasin-like substances were demonstrated in tissues of Gaucher's disease. Frozen sections were completely dehydrated and defatted in successive changes of acetone followed by petrolic ether. Drops of quinoline were added to the slide. Mild heating, followed by cooling, permitted the crystallization of kerasin-like substances. A selenite plate of the first order was placed between the slide and the substage condenser of the polarizing Nicol's prisms. Against the salmon-red to pink background, Maltese crosses and, under higher magnification, hexagonal crystals with blue and yellow crossed segments became visible.

A brief résumé of the current status of the lipoid substances in Gaucher's disease was given.

**FILARIASIS IN AMERICAN ARMED FORCES.** William B. Wartman, Cleveland, O.

**Abstract.** This report is based on a study of material collected at the Army Institute of Pathology from cases of filariasis contracted during World War II. It consisted of 63 specimens taken for biopsy from military personnel and included 57 lymph nodes, 3 spermatic cords, and 3 epididymides. Accurate information about the epidemiology of the disease was available. About 90 per cent of patients were infected in the Samoan Islands, 4 per cent in the Solomon Islands, and the rest in the South Pacific area. One case was apparently infected in New Guinea. The average length of exposure in endemic areas was 11 months, with extremes of 1 to 30 months. The incubation period was between 5 and 15 months in most cases and the earliest proved case was 3 months.

Clinical findings consisted of genital lesions, acute lymphangitis of the extremities, especially the arms, lymphadenitis, and peculiar fugitive swellings. Constitutional manifestations were slight or absent; the attacks lasted a few days, and recurrences were the rule. Genital lesions included acute funiculitis, epididymitis, orchitis, inflammation of scrotal skin, and hydrocele. Lymphangitis was commonly manifested by raised, tender, red streaks and spread centrifugally. Lymphadenitis often preceded the lymphangitis. Microfilariae were absent from the blood in the great majority of cases, but were found in very small numbers on one occasion in each of 7 patients. Skin tests with *Dirofilaria immitis* antigen were positive in about 90 per cent of cases. Cultures of lymph nodes, blood, and hydrocele fluid were negative.

Tissue reactions consisted of acute and chronic granulomatous inflammation, proliferation of cells of the reticulo-endothelial system, and exudation of eosinophils. When adult worms were present, typical granulomas formed around them and there was striking proliferation of littoral cells and reticulum. In other portions of the node there was hyperplasia of lining cells of sinuses which often appeared as a tongue-like process growing into the distended channels. Eosinophils were abundant and sometimes there were "eosinophilic abscesses." Eventually, adult worms either disappeared or were encapsulated with collagenous connective tissue, or became calcified. Microfilariae were found in some specimens, but it was difficult to determine whether the accompanying tissue changes were due to microfilariae or to an allergic reaction to a nearby or distant adult worm.

**Discussion**

(Dr. Howard T. Karsner, Cleveland, O.) Dr. Wartman did not have time to refer to the diagnostic value of biopsy in filariasis. In this connection, the main question is whether, in those lymph nodes in which worms or microfilariae are not identified, the presence of granulomas and histo-eosinophilia is sufficient to justify a positive diagnosis.

(Dr. Wartman) In reply to Dr. Karsner's question I firmly believe that the only positive way of making a diagnosis, particularly in the occasional case, is by identification of the infecting organism, either adult worms, or the microfilariae. However, in cases in which there is known to be exposure to the disease, and in which there are clinical symptoms present, the finding of the other changes is confirmatory of the diagnosis.

**THE SERODIAGNOSIS OF AMEBIASIS: EVALUATION OF THE CURRENTLY AVAILABLE ANTIGENS IN A QUANTITATIVELY STANDARDIZED COMPLEMENT-FIXATION TEST.**

John F. Kent (by invitation) and Charles R. Rein, Washington, D.C.

*Abstract.* A complement-fixation test employing quantitative criteria in the standardization of reagents and test conditions has been applied in the evaluation of antigens for the serodiagnosis of amebiasis. Results obtained in experimental studies with serums from established cases of amebiasis, subjects with other diseases, and presumed normal persons indicate the advantages inherent in the quantitatively standardized procedure, and point out the limitations in sensitivity and specificity of the available antigens. Current studies directed toward improvement of the test are discussed.

**ACUTE MALARIAL LESIONS PRODUCED IN CHICKS BY PLASMODIUM GALLINACEUM.**  
Lloyd R. Hershberger and G. Robert Coatney (by invitation), Bethesda, Md.

*Abstract.* Eight-day old chicks were inoculated intravenously with  $16 \times 10^6$  parasites. (Blood-induced infection.) The acute lesions in the major organs were then followed by tissue studies on chicks sacrificed on succeeding days. Malarial pigment deposits were present in the spleen, liver, bone marrow, kidney, heart, and brain, with variable disorganization caused thereby. Acute inflammatory lesions were noted in the spleen, lungs, and pancreas. The bone marrow showed moderate hyperplasia. Extramedullary hematopoiesis was seen in the liver, heart, and lungs. Slight fatty changes were present in the liver, heart, and kidneys. Exo-erythrocytic parasites were most frequently seen in the brain in the late stages of the acute infection.

*Discussion*

(Dr. R. H. Rigdon, Little Rock, Ark.) These pathologic changes described by Dr. Hershberger are very interesting. I would like to know whether his birds developed a severe anemia, because in following the pathologic changes in ducks with malaria we found that the characteristic process is a progressive anemia. We feel this is one of the important things in the mechanism of the pathologic changes and ultimate death.

I would like to ask if Dr. Hershberger looked for necrosis around the central veins of the liver. His comment with regard to edema of the heart muscle is interesting, due to the fact that we feel there is a circulatory failure based on the acute anemia in the duck. I wonder if that might not also occur in the chick. I wonder if there were any pathologic changes looked for specifically in the brain, since we have already described numerous degenerative changes which occur in acute malaria in the brain.

(Dr. Israel Davidsohn, Chicago, Ill.) How long was the interval between the inoculation and the death of the animal in those cases where there were extramedullary foci of hematopoiesis?

(Dr. Robert J. Parsons, Naval Hospital, Great Lakes, Ill.) I would like to ask if the exo-erythrocytic forms were not present in the other organs which Dr. Hershberger described. He mentioned them only in the brain. In my experience they occur in all organs of the body.

(Dr. Hershberger) In reply to Dr. Rigdon, we did not find any other lesion of the brain in these birds. Concerning anemia, the bone marrow showed evidence of hyperplasia in both the erythroid and myeloid series. Concerning foci of extra-medullary hematopoiesis, we did not notice any difference in the survival of birds with or without extramedullary hematopoiesis. Almost uniformly there was extra-medullary hematopoiesis in our birds. Concerning the exo-erythrocytic forms in other organs, our paper was intended to deal with the pathologic changes rather than the parasites in this disease.

(Dr. Davidsohn) The question was—how long was the interval between the inoculation and the death of the animals in those animals where there were extra-medullary foci of hematopoiesis?

(Dr. Hershberger) The normal birds at this stage show extramedullary foci of hematopoiesis, and what we had was an increase; there was no significant difference between the chicks which had a lot of extramedullary hematopoiesis and those which did not.

**RELATIVE ACTIVITY OF SULFONAMIDES AGAINST DYSENTERIC BACILLI AND THEIR TOXIC FILTRATES.** F. J. Moore (by invitation) and J. Marmorston, Los Angeles, Calif.

**Abstract.** Growth-inhibiting concentrations of sulfathiazole, sulfadiazine, sulfaguanidine, and sulfasuxidine were determined *in vitro* for 38 strains of *Salmonella-Shigella* organisms. The first 2 were further examined against 83 strains. It was found that sulfathiazole was 2.5 times as active as sulfadiazine, 40 times as active as sulfaguanidine, and 100 times as active as whole sulfasuxidine (unhydrolyzed) against these bacteria on the average. Each of these four drugs was tested for *in vivo* activity against toxic Flexner filtrates administered intraperitoneally to mice or intravenously to rabbits. No protective or therapeutic action was noted. Conclusions were drawn as to the site requiring treatment in dysentery.

**INTRACRANIAL NEOPLASMS PRODUCED IN DOGS BY METHYLCHOLANTHRENE.** R. M. Mulligan and K. T. Neubuerger, Denver, Colo.

**Abstract.** The implantation of methylcholanthrene in 100 mg. doses in gelatin capsules beneath the dura over the right frontoparietal area of the cerebrum of 7 young male mongrel bulldogs resulted in the production of intracranial neoplasms in 3 dogs. These neoplasms were an extracerebral fibrosarcoma, a glioma with the histologic characteristics of a spongioblastoma polare, and a sarcomatous meningioma, observed after 378, 444, and 436 days respectively.

*Discussion*

(Dr. Percival Bailey, Chicago, Ill.) Some years ago one of my pupils placed capsules of methylcholanthrene in the cortex of several dogs. The carcinogen was inserted directly into the cortex. It is very easy to produce tumors with carcinogens from the meninges of dogs. All of these animals developed convulsions after 6 to 8 months or so without the carcinogenic agent being anywhere near the motor cortex. Besides that, there was no reaction on the part of the neuro-epithelial tissue, and I would have to be convinced that the tumor reported here as a spongioblastoma was of neuro-ectodermal origin. I say that because in other cases I have not been able to convince myself that the tumors reported were really of neuro-epithelial origin. Dogs develop sarcomas of the brain spontaneously and rather frequently. Moreover, it has proved difficult to produce gliomas in the brains of animals, except in those of one particular strain of mice. I feel, therefore, that conclusive evidence is necessary before this tumor is labelled spongioblastoma polare.

(Dr. Mulligan) We studied these tumors with a number of different stains. With the hematoxylin and eosin stain there was a pronounced difference between the fibrosarcoma and the spongioblastoma. The nuclei in the fibrosarcoma had rather small basophilic nucleoli, and on the average they were much shorter than in the tumor which we think is a spongioblastoma. In the spongioblastoma the nuclei were much longer and there were eosinophilic nucleoli. There was a distinct difference in the chromatin pattern as well. I also mentioned the absence of any reticular fibrils in the glioma with the reticulum stain, whereas the fibrosarcoma contained many such fibrils. If you do not mind, I think that Dr. Neubuerger would be happy to send you slides to see what you make of these tumors yourself.

(Dr. Bailey) I would be glad to examine them.

(Dr. Howard T. Karsner, Cleveland, O.) This should be incorporated into the record.

(Dr. Bailey subsequently examined sections of these tumors and stated: "I believe all three of the tumors to be of connective tissue nature and that none of them is neuro-epithelial.")

**STUDIES ON CAPILLARY PERMEABILITY AS Affected BY ANOXEMIA.** Howard C. Hopps, Oklahoma City, Okla. (by invitation) and Julian H. Lewis, Chicago, Ill.

*Abstract.* The minimum latent period for anaphylactic shock in guinea-pigs following passive sensitization is presumed to be an indication of the time necessary for antibodies to escape from the blood stream and into the tissues. This minimum latent period was not shortened as the result of acute anoxemia brought about by subjecting passively sensitized guinea-pigs to low oxygen tensions. Therefore, anoxemia, under these conditions, does not facilitate the passage of antibody globulin through vascular endothelium.

Studies on the rate of disappearance of T-1824 from the blood stream indicate that acute anoxia acts to *decrease* slightly the normal rate of disappearance of this dye. As an explanation of this phenomenon it is suggested that (a) anoxia, under these conditions, does not facilitate the passage of albumin-T-1824 complex through vascular endothelium and (b) anoxia acts to inhibit the mechanism by which T-1824 normally leaves the blood stream.

Significant alterations in the quantity of serum protein following acute anoxia produced under the conditions of the experiment were not observed.

**PARENCHYMATOUS DEGENERATION RELATED TO ANOXIA.** Virgil H. Moon, Philadelphia, Pa.

*Abstract.* Acute parenchymatous degeneration is commonly attributed to toxic effects, as in severe infections, poisoning, and intoxications, both endogenous and exogenous. But it develops also under other conditions, particularly when anoxia is a factor in causing death. In observations on the pathology of shock, parenchymatous degeneration was found regularly. This was noted in clinical cases of shock from trauma, surgery, burns, and in experimental shock produced by various means. Degenerative changes were especially marked in the kidneys and liver, less so in the myocardium. In cases of trauma and burns, toxic substances absorbed from damaged tissue might be suggested as causing parenchymatous degeneration. However, there are instances in which no toxic effect seems possible. Acute parenchymatous degeneration was found after death by coronary occlusion, nitrous oxide anesthesia, suffocation, heat stroke, anaphylaxis, and after shock from high-altitude aviation. Anoxia seems to be the common denominator in the various conditions mentioned and should be recognized among the causes for parenchymatous degeneration.

*Discussion*

(Dr. Joseph Tannenberg, Batavia, N. Y.) I would like to ask whether Dr. Moon has seen, in these cases, focal necroses in the liver and eventually in the heart. During 1936 to 1938 I studied, in quite extensive experiments on rabbits, what I then called anoxic shock. Short periods of intensive anoxia were produced under simultaneous elimination of carbon dioxide in order to produce conditions which were purely due to the anoxia. In these rabbits I saw quite a good many focal necroses within the liver and within the heart, the skeletal muscles, and the brain. I have not seen, as far as I can remember at this time, anything that looked like focal necrosis in the kidneys.

(Dr. Howard T. Karsner, Cleveland, O.) In connection with functional disturbances, I wish to refer to the brief report by E. E. Selkurt which appeared in *The Clinical Bulletin of the School of Medicine of Western Reserve University and Its Associated Hospitals*, 1945, 9, 87-94. This was based on production of complete renal ischemia and on hemorrhagic shock. In both, there was widespread nephrosis in the kidneys (dogs) which were examined microscopically, evidently the result of local anoxia. The results indicated that the renal tubular excretory mechanism is impaired and that there is almost complete resorption of tubular fluid and its contained substances, the injured tubular cells losing their normal selectivity.

(Dr. C. V. Weller, Ann Arbor, Mich.) If Dr. Moon found that the cases with traumatic shock, which he used, were complicated by massive pulmonary fat embolism, that fact would explain the anoxemia. I do not think he made it clear whether that possibility was investigated.

(Dr. Norbert Enzer, Milwaukee, Wis.) I would like to state also for the record and as a tribute to a great member of this society, Dr. Oscar Schultz, that more than 20 years ago he spoke of the kidney of low oxygen. Time and again, in the laboratories, he called attention to precisely the changes reported here today. He commented also on the fact that kidneys removed at operation, particularly those in which the operative procedure was rather difficult and prolonged, frequently showed the pathologic changes in the renal tubules which have been referred to here. This subject has an important practical significance for those engaged in medicolegal pathology, in differentiating death from shock due to accident as against death from nontraumatic causes, where recognition of these changes may be effective in establishing the cause of death.

(Dr. Alex B. Ragins, Chicago, Ill.) I would like to ask in the case of the high-altitude deaths whether or not the changes in the liver were similar to those I have observed in cases of airmen who, returning from their high-altitude run, reported being well over the telephone communications system and 15 minutes later were found to be dead in the plane. On microscopic examination their livers showed peculiar vacuolization in the liver cells. There were neither fat nor glycogen-containing vacuoles. I wonder if Dr. Moon noticed that in his cases, and what they might be?

(Dr. Moon) I am deeply gratified by the interest that has been shown in this presentation. I can only comment briefly on the suggestions that have been made.

There were very marked changes in the liver in these cases as well; sometimes it was in the nature of focal necrosis; in other instances scattered groups of cells or individual cells were necrotic. I found focal necrosis rarely in the renal cortex, but rather necrosis of individual cells or groups of cells, as shown in the photomicrographs, in various parts of the tubules.

Pulmonary fat embolism was possible in cases of severe trauma. Unfortunately we did not have opportunity to test for fat in the pulmonary capillaries because the cases came from the Army Institute of Pathology and only stained sections were available. In other instances there appeared no opportunity for pulmonary fat embolism; deaths from coronary occlusion, nitrous oxide anesthesia, anaphylaxis, or from other causes probably were not complicated by fat embolism.

In high-altitude shock, of which 5 cases were examined, there was very marked degeneration of the liver as well as of the kidney. Had there been time, it would have been interesting to show corresponding sections of the liver and the kidney in each case, showing parenchymatous degeneration ranging in degree to necrosis of the cells.

THE INFLUENCE OF AGE AND SPECIES ON THE NEPHROTOXIC ACTION OF DL-SERINE.  
Robert P. Morehead and (by invitation) W. D. Poe, J. O. Williams, and M. E. Lazenby, Winston-Salem, N. C.

*Abstract.* In previous experiments it has been shown that *dl*-serine exerts a pronounced nephrotoxic action in white albino rats weighing 100 gm. When animals of this type are placed on an experimental diet supplemented with *dl*-serine and deficient in the B vitamins, a high mortality follows, and the changes in the kidneys are more severe than in animals receiving the amino acid and maintained on a stock diet considered adequate. Addition of the B vitamins to the experimental diet reduces the mortality greatly but does not alter the renal lesions.

Young guinea-pigs and rabbits were placed on an experimental diet deficient in the B vitamins, and *dl*-serine was administered daily. A high mortality resulted, but no lesions were demonstrable in the kidneys. A group of mature mice were maintained on the deficient diet and given *dl*-serine daily. No deaths occurred in this group, nor were lesions demonstrable in the kidneys. Young mice are at present being employed under the same experimental conditions.

*Discussion*

(Dr. E. T. Bell, Minneapolis, Minn.) I would like to point out that something may be learned about how a toxic substance acts upon the kidney if one ureter is ligated. Ligature of the ureter suppresses glomerular filtration on that side. For example, if a ureter is ligated 24 hours or more before injection of bichloride of mercury or uranium nitrate, there is no injury to the hydronephrotic or obstructed kidney. On the other hand, sucrose produces great changes in the obstructed kidney, as in the normal. Evidently, substances like bichloride and uranium nitrate are concentrated in the tubule and produce their changes in that way.

(Dr. Paul R. Cannon, Chicago, Ill.) I would like to ask if this effect of *dl*-serine was produced by any of the other amino acids.

(Dr. Morehead) Many amino acids have been found to be nephrotoxic, particularly when injected. This action appears, however, to be confined for the most part to very young, growing animals. The changes in the kidney following the administration of these substances have more closely resembled those of acute cortical necrosis than the changes resulting from *dl*-serine. I might add further that in recent experiments we have found that *l*-serine is not nephrotoxic.

INFLUENCE OF EXPERIMENTAL RENAL DAMAGE ON HISTOCHEMICALLY DEMONSTRABLE LIPASE ACTIVITY IN THE RAT. COMPARISON WITH PHOSPHATASE ACTIVITY. Max Wachstein, Middletown, N. Y.

*Abstract.* The lipase activity of the kidneys of different animals was examined with the microtechnic of Gomori (*Proc. Soc. Exper. Biol. & Med.*, 1945, 58, 362-364) with some modifications. Preparations stained for alkaline phosphatase were also made. The rat was found to have a very constant lipase activity restricted to the cytoplasm of the cells composing the proximal convoluted tubules. After poisoning with uranium nitrate as well as mercury bichloride, the enzyme was not inactivated in necrotic and destroyed cells. However, there was a marked decrease

of its amount in the cells of regenerating and atrophic tubules. In rats in which severe hemorrhagic necrosis was produced by means of dietary choline deficiency, there was depletion of the enzyme in the necrotic cells in the acute stage and marked deficiency of the enzyme in the kidneys of animals surviving the acute damage. After ligation of the ureter, marked depletion of the enzyme occurred wherever the hydronephrotic changes were prominent. Alkaline phosphatase activity showed quite similar changes under the experimental conditions mentioned above. Wherever lipase activity in the convoluted tubules was diminished, this was found to be true also for the phosphatase activity in the cytoplasm of cells in this location. The significance of lipase and phosphatase activity in the convoluted tubules is not definitely clarified. Cellular damage, however, influences both enzymes in a very similar manner.

#### *Discussion*

(Dr. G. Gomori, Chicago, Ill.) I would like to ask whether you have demonstrated lipase in man, either in normal or pathologic conditions. I have about twenty-five sections of normal and abnormal human kidneys and I have never found even traces of lipase.

(Dr. Wachstein) Neither in surgically removed kidneys nor in very fresh post-mortem material could lipase activity be found in tissue sections. However, in view of the fact that this enzymatic activity depends to a large extent on the substrate used and that lipase is found by biochemical methods in human kidneys, it is hoped that by applying a suitable substrate, lipase activity will also be demonstrated in tissue section.

(Dr. H. Edward MacMahon, Boston, Mass.) I would like to ask if one sees the return of this material during repair as rapidly as one sees its histologic disappearance in the early stages of disease.

(Dr. Wachstein) Wherever cells show loss of enzymatic activity they are also different in sections stained with hematoxylin and eosin. These morphologically changed cells did not show a return of enzymatic activity during the time the experiments were carried out.

#### **EXPERIMENTAL STREPTOCOCCAL INFECTIONS OF THE CHORIOALLANTOIC MEMBRANE OF THE EMBRYONIC CHICK. Noble P. Sherwood, H. R. Wahl, Catherine Colglazier (by invitation), and Tom R. Hamilton, Lawrence, Kan.**

*Abstract.* This paper contains a comparison of the pathologic changes and host-parasite relationships of 24 strains of hemolytic streptococci representing the Lancefield groups A to K. The results reveal a striking difference in invasive property, necrosis of ectoderm and mesoderm, ability to spread within the mesoderm and to multiply within phagocytic cells of the mesoderm. The 6 strains from cases of endocarditis, while more invasive than many other strains, exhibited wide differences in ability to spread and find host cells within the mesoderm. No correlation between virulence and hemolysins for chicken cells was observed.

#### **MENINGOCOCCIC PURPURA AND THE SHWARTZMAN PHENOMENON: AN EXPERIMENTAL STUDY. B. Black-Schaffer and (by invitation) G. P. Kerby and T. G. Hiebert, Durham, N. C.**

*Abstract.* The Schwartzman phenomenon (largely by means of bacterial filtrates) has been the method of demonstrating the presence of a purpurogenic factor in many strains of meningococci. The time required to produce the phenomenon closely parallels the interval between the first symptoms and the appearance of the cutaneous purpura in many instances of clinical purpuric meningococcemia. The

unexplained selectivity of the human disease finds its analogue in the widely differing response of individual rabbits to the same dose of Shwartzman substances.

Because of these observations, and in order to more closely simulate human purpuric meningococcemia, the investigation here reported was undertaken. Living washed organisms recovered from an instance of purpuric meningococcemia were used throughout. It was discovered that after two washings with normal saline solution the organisms possessed the ability to prepare (intradermal inoculation) the skin of rabbits and elicit (intravenous inoculation) the phenomenon without the agency of the usual potent filtrates. The same properties were demonstrated by heat-killed, washed meningococci. In contrast to Shwartzman's experience, the washed organisms were more potent than the homologous supernatant fluid.

It is concluded that purpuric meningococcemia suggests a spontaneously occurring Shwartzman phenomenon; the organisms present in the purpuric lesions may be the counterpart of those intradermally inoculated into the rabbit. The meningococcemia, then, probably represents the provocative factor, just as do the organisms intravenously administered in the experiment.

#### *Discussion*

(Dr. John R. Schenken, Omaha, Neb.) How does the essayist account for the appearance of purpura in cases within an hour or two after the onset of the disease? We reported a case where the child was perfectly well at 4 o'clock in the afternoon and dead at 7:30 P.M., having developed purpura in that period of time.

(Dr. Joseph Tannenberg, Batavia, N. Y.) I would like to ask whether there were hemorrhagic infarctions of the adrenals also, in the experiments reported, as is the case frequently in patients dying of the Waterhouse-Friderichsen syndrome.

(Dr. Black-Schaffer) In response to the first question, it seems to me that in those cases which I have observed the patients are usually ill with a respiratory infection a day or two before the onset of the disease. The organisms are capable of getting into the skin during the course of the prodromal syndrome. In animals, all we need is a matter of 2 hours, once the organisms invade the blood stream, to produce the phenomenon. After that time the lesions manifest themselves as small cyanotic areas which then become purpuric and eventually develop a necrotic center; so that, while I cannot give you an authoritative answer, I think that the experiment appears capable of explaining the appearance of purpuric spots only a few hours after the apparent onset of the disease, which corresponds to the meningococcemia.

In reply to the second question, I hesitate to suggest that the adrenal hemorrhages are solely explicable on the basis of the Shwartzman phenomenon. They may occur in experimental animals after one intravenous inoculation of the substance, and I think that this may represent something similar to the hemorrhage-producing ability of the Shwartzman substance in tumors, where it is possible to destroy some forms by one intravenous inoculation. We would expect to find more patients with the massive type of adrenal hemorrhage were it produced by the mechanism of the usual Shwartzman phenomenon.

#### **HISTOPATHOLOGIC STUDY OF ANAPHYLACTIC SHOCK IN IDENTICAL TWINS.** Jacob Werne and (by invitation) Irene Garrow, New York, N. Y.

**Abstract.** Histologic study in identical male twins, 10 months of age, dying of delayed anaphylactic shock (16 and 20 hours after their second immunizing injection of diphtheria toxoid and pertussis antigen) disclosed visceral changes, of which the most prominent feature was vascular injury. The lesions encountered were similar in both cases, with minor variations. There was thickening of small

arterial walls, with extreme luminal narrowing; endothelial degeneration and early proliferation; exudation of fluid into vessel walls and about capillaries; necrosis of occasional arteries, with young thrombi; widespread capillovenous engorgement, with focal hemorrhages; and acute degeneration of parenchymal cells in brain, heart, lung, liver, adrenal, and lymphatic tissue. Tissue eosinophilia was most marked in bone marrow and thymus; conspicuous intravascular eosinophils were noted in some cerebral and pulmonary vessels, and in hepatic sinusoids. Narrowing of some bronchial and bowel lumina was evident. The follicles of the spleen, lymph nodes, and intestines showed marked phagocytic activity. Large amounts of ingested nuclear débris were present at their centers. Pulmonary emphysema was not present, indicating that the small amount of bronchoconstriction noted histologically was unrelated to the fatal outcome. Engorgement of the liver was extreme and was the most conspicuous gross finding in one twin; in the other, focal pulmonary hemorrhages, which on microscopic examination showed early bronchopneumonic consolidation, constituted the only distinctive gross finding.

The post-mortem investigation excluded contamination of the biological therapeutic agents, and of detectable disease. Attempts at passive transfer, using post-mortem serum, were unsuccessful. There was a history of angioneurotic edema in the father. The widespread morphologic appearances are interpreted as reflecting the known effect of the antigen-antibody reaction upon the smooth muscle and vascular endothelium of the anaphylactic body. In the cases presented the pathologic picture is uncomplicated by associated therapy or concurrent illness. It contributes further evidence of the basic rôle of vascular injury in the hypersensitive state.

#### *Discussion*

(Dr. Edmund Mayer, Stamford, Conn.) I wonder what emphasis has been placed in your investigation on the fact that these infants happened to be twins. What was the evidence that they were identical twins? Do you think that the distribution and quality of the lesions were more similar in those twins than one would expect to find in two infants of the same age who died from the same serum, but were not identical twins?

(Dr. Werne) The fact that the twins reacted in an identical manner to the second injection of a product which, so far as we have been able to determine, has not resulted in a fatality, is in conformity with our knowledge of the biologic similarities exhibited by identical twins. There were minor variations among the lesions encountered, such as more extensive hepatic congestion in Gary, and the presence of foci of hemorrhagic bronchopneumonia in Donald. The diffuse vascular lesions with their associated parenchymal degenerative changes were alike in both. Incidentally, the physician who gave the immunizing injection was the one who delivered the twins. He stated that they had a common placenta.

(Dr. B. Black-Schaffer, Durham, N. C.) Was the Prausnitz-Küstner reaction carried out to demonstrate sensitivity?

(Dr. Werne) Attempts at post-mortem transfer were made using four volunteers among the laboratory staff. This was not successful. Dr. Jules Freund of the Bureau of Laboratories of the New York City Health Department, who made exhaustive studies on the product in order to exclude primary toxicity and contamination, attempted previous transfer, using post-mortem serum, to guinea-pigs, also without success. According to the available literature, there is no constant relationship between the clinical response and the demonstrable reagin. Walzer reported several cases in which, at the height of the anaphylactic shock, previous transfer was unsuccessful; following recovery he was successful in demonstrating reagin. We are familiar, of course, with the success of Lund and Hunt in demonstrating the Prausnitz-Küstner reaction in post-mortem serum from their case of instantaneous anaphylactic death.

THE NONPORTAL DISTRIBUTION OF THE TRABECULAE IN DIETARY CIRRHOSIS OF RATS AND CARBON TETRACHLORIDE CIRRHOSIS OF RATS AND GUINEA-PIGS.\*  
L. L. Ashburn, K. M. Endicott, F. S. Daft (by invitation), and R. D. Lillie, Bethesda, Md.

*Abstract.* The purpose of this study was to obtain further information on the relationship of the trabeculae to the portal and hepatic veins in certain experimental cirrhoses. Albino rats at weaning were placed on a low-protein, choline-deficient diet, and killed after 50 to 150 days. The livers were injected through the portal or hepatic veins with a charcoal gelatin mass to effectively mark these structures in the microscopic preparation. Histologic study showed that the fatty deposition, ceroid accumulation, and fibrous trabeculation primarily followed and connected hepatic veins. In livers showing marked alteration, the trabeculae sometimes included, coursed by, or abutted on large portal areas. However, even in these livers the portal areas comparable in level to the centro-lobular veins were not primarily related to the trabeculae. In other experiments, cirrhosis in rats and guinea-pigs was produced by the repeated subcutaneous administration of carbon tetrachloride. The livers were injected with the charcoal gelatin mass and studied histologically. The connective tissue trabeculae occurring in these livers were primarily related to hepatic veins and showed essentially the same distribution as that seen in dietary cirrhosis.

HEMOPOIESIS IN FOLIC ACID AND RIBOFLAVIN DEFICIENCY. K. M. Endicott and (by invitation) A. Kornberg and M. Ott, Bethesda, Md.

*Abstract.* Folic acid deficiency occurs in rats given a purified diet containing sulfasuxidine. This results in pancytopenia and especially granulocytopenia. Similar blood dyscrasias develop in rats fed a diet deficient in riboflavin. Comparative quantitative studies of hemopoiesis in these two deficiencies were made. In both deficiencies there is a progressive depletion of all types of cells of the granulocytic series in the bone marrow. There is a less marked depletion of the erythrocytic and megakaryocytic series. The spleen becomes atrophic with small inactive follicles and no hemopoiesis. The thymus involutes and lymph nodes become atrophic. The lymphopenia, granulocytopenia, and anemia of folic acid deficiency are spectacularly remedied by daily oral doses of crystalline folic acid. This is accompanied by a marked regeneration of bone marrow, splenic hemopoiesis, and increased size and activity of the lymphoid apparatus. The lymphopenia and granulocytopenia of riboflavin deficiency respond somewhat more slowly to crystalline folic acid therapy and this slower response is also noted in the bone marrow, spleen, and lymphoid apparatus. The anemia of riboflavin deficiency responds to riboflavin therapy but not to folic acid therapy.

BLINDNESS IN DUCKS ACCOMPANYING HYPOGLYCEMIA.\* R. H. Rigdon and (by invitation) D. E. Fletcher, Little Rock, Ark.

*Abstract.* Ducks given large amounts of insulin develop a marked hypoglycemia and, accompanying this decrease in sugar, the birds lose their sight. This loss of vision is a temporary change which persists as long as the sugar level is abnormally low. Sight returns when the sugar returns to normal amounts. This change apparently results from a disturbance in cerebral glycolysis. Acute degenerative changes occur in the optic nerve and the brain of these birds.

*Discussion*

(Dr. Joseph Tannenberg, Batavia, N. Y.) Six years ago I presented before the meeting of this Association a paper with the title, "Anatomical Changes Produced by Short Periods of Anoxia (Anoxic Shock). The Effect of Frequent Repetitions

\* This article will appear in a subsequent issue of *The American Journal of Pathology*.

and Combinations with Insulin" (*Am. J. Path.*, 1940, 16, 656-657). I went the other way around and studied first what I called anoxic shock. In this condition intensive anoxia was applied until the animals were seized by convulsions or the respiration stopped. In these experiments we found an absolutely identical symptomatology in both types, anoxic and insulin shock, and the same anatomic changes that were demonstrated today. Various degenerative lesions were produced throughout the brain. Since these shocks were frequently repeated, on some animals 20 to 30 times, we found many transitional stages between fresh and older lesions. Particularly well demonstrable were lesions in the cerebellum, affecting the Purkinje cells, many of which were degenerated. I do not know whether these rabbits became blind, since they were kept in cages, but some might have been.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) I would like to ask Dr. Rigdon whether any attempt was made to weigh the brains of the ducks which died in the acute stage of shock. The most impressive findings in the cases of fatal insulin shock in men, as reported 9 years ago at the Chicago meeting (*Am. J. Path.*, 1937, 13, 664-666), was a considerable increase in the weight of the brain from true swelling. Obviously the sensitivity of the various parts of the brain in ducks is different from that in the human brain. In the human material examined by me the most severe changes were in the cortex. In contrast, there was comparatively little damage in the brain stem. These marked changes in the cortex were identical with changes seen in severe cases of swelling of the brain from various causes (including oleum Chenopodium poisoning, avertin anesthesia with cessation of breathing, and severe burns). We explained them on the basis of anoxia rather than as in any way specific for hypoglycemia.

(Dr. Tannenberg) The physiologic basis which explains why anoxic and insulin shock have the same symptomatology is this: According to the work of Himwich, the brain cells can live only on carbohydrate and on nothing else. Therefore, for their metabolism two things are required: blood sugar and oxygen. The same effect should be obtained when either of them is deficient.

(Dr. Rigdon) In answer to Dr. Terplan's question about the weight of the brain in these ducks, I can only state that we did not weigh any of them. It is of interest to observe these lesions in the optic tracts, brain stem, and cerebellum of ducks while similar lesions occur in the cortex in man. Of course the duck does not have a cerebral cortex.

#### READ BY TITLE

#### OBSERVATIONS ON THE CULTIVATION OF BACTERIUM TULARENSE IN EMBRYONATED EGGS. Lewis L. Coriell, Capt., M.C. (by invitation), Cora M. Downs, Gifford B. Pinchot, Lt., U.S.N.R. (by invitation), Elizabeth Smadel, and (by invitation) Alice Klauber, Lt., U.S.N.R., Lawrence, Kan.

*Abstract.* Studies using a measured number of virulent *B. tularensis* as inoculum in embryonated eggs showed that the organisms grew more abundantly in the cells of the yolk sac than in the chorioallantoic membrane or in the embryo. The organisms grew equally well in duck eggs. Strains of *B. tularensis* of lowered virulence grew less vigorously than virulent strains, whereas the completely avirulent strain 38 did not survive or multiply in embryonated eggs. *B. tularensis* also grew well in eggs in which the embryo was killed at the time of inoculation. Serial passage of virulent strains did not alter their virulence for mice or the chick embryo. The structural changes produced by *B. tularensis* in the embryonated egg are described.

#### GENERALIZED BOECK'S SARCOIDOSIS WITH THROMBOCYTOPENIC PURPURA. Norbert Enzer, Milwaukee, Wis.

*Abstract.* The patient was a man, 32 years of age, who was observed for a period of 3 years. His first complaints were considered suggestive of peptic ulcer, although

at that time hydrochloric acid was absent from the stomach and roentgenograms failed to reveal an ulcer. He continued to have intermittent abdominal pain, for which an appendectomy was finally performed. The appendix showed only mild inflammation. Roentgenograms of the chest about 2 years before death disclosed fibrosis of both upper lobes. A tuberculin test was negative repeatedly and gastric washings were likewise negative for tubercle bacilli. About 8 months before death he developed a severe anemia, thrombocytopenia, leukopenia, and purpura. Examination of the bone marrow showed normal hyperplasia. All efforts to relieve the hematopoietic situation failed and finally, with the purpura becoming more severe, a splenectomy was performed. The spleen was enlarged and nodular and filled with lesions typical of Boeck's sarcoid. Death occurred from postoperative hemorrhage. Autopsy disclosed sarcoidosis of the lungs and lymph nodes. No lesions of Boeck's sarcoid were found in the bones or bone marrow.

**EXTENSIVE DESTRUCTION OF THE BRAIN IN ECLAMPSIA.** Herman Josephy and Edwin F. Hirsch, Chicago, Ill.

*Abstract.* Eclamptic convulsions in a young woman were followed by vegetative existence until death 3 months later. The brain had extensive regions of destruction in various stages of organization. There are only a few descriptions of this condition in the literature.

**BILATERAL ACUTE HEMORRHAGIC NECROSIS OF THE ADRENALS IN A YOUNG CHILD.  
(A CASE OF WATERHOUSE-FRIDERICHSEN SYNDROME.)** Joseph Tannenberg, Batavia, N. Y.

*Abstract.* In a rural area of western New York about 5 cases of meningococcal infections, most of which were cases of meningitis, were seen annually in the past 5 years. In 3 cases a meningococcal septicemia was present. Two of them showed multiple petechiae in the skin. All recovered under treatment with sulfadiazine and, later, penicillin. In a neighboring county we have seen recently a case of a child of 5 years who, on the second day of what appeared to be a slight cold, died in convulsions on the way to the hospital. The autopsy showed nothing but a slight hyperemia of the leptomeninges! Only histologically was it possible to make the diagnosis of a fresh meningococcal meningitis, with recovery of meningococci. There was no involvement of the adrenals. Quite in contrast to these cases stands a case that presented the typical Waterhouse-Friderichsen syndrome. A child in a good state of nutrition and previously in good health suddenly was taken ill with fever up to 102°F. No changes in the lungs were shown roentgenologically. During the first day of his illness multiple large petechiae developed in the skin. The child died 2 hours following the first administration of 20,000 units of penicillin. At autopsy a bilateral complete hemorrhagic infarction of the adrenals was found. Blood taken from the right ventricle remained, on culture, sterile for meningococci. One tonsil cultured yielded a hemolytic staphylococcus which gave a positive coagulase test. Histologically, there was no evidence of meningitis; practically no other changes were found except partial necrosis of lymph follicles in the spleen and large secondary follicles in the tonsils. Here a number of cocci were seen within phagocytic cells. These cocci were single and Gram-positive. No diplococci were recovered. A few days before the child was taken sick the mother had tonsillitis which, a week after the death of the child, led to the development of otitis media. It is held that bilateral hemorrhagic infarction of the adrenals may occur in rare instances of septicemia, particularly in those caused by the meningococcus. However, it is not held that this anatomic picture is absolutely diagnostic for meningococcal infections, particularly when it is considered that even in the largest series of 19 cases, which were reported from a single institution, it had been possible only in 6 cases to recover meningococci from the blood and organs of the stricken person.

